PETITION TO LIST THE LARGE MARBLE BUTTERFLY *Euchloe ausonides* (Lucas, 1852) AS A THREATENED SPECIES

AND THE LARGE MARBLE TYPE SUBSPECIES *Euchloe ausonides ausonides* (Lucas, 1852) AS AN ENDANGERED SPECIES

UNDER THE U.S. ENDANGERED SPECIES ACT

Euchloe ausonides, Jackson County, Oregon. Photograph: Ken Kertell, iNaturalist, used under Creative Commons license.

Submitted by The Xerces Society for Invertebrate Conservation

Prepared by Kevin Burls, Emily Ruth Spindler, and Candace Fallon

5 October, 2023

NOTICE OF PETITION

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PETITIONER

The Xerces Society for Invertebrate Conservation is a nonprofit organization that protects wildlife through the conservation of invertebrates and their habitat. For fifty years, the Society has been at the forefront of invertebrate protection worldwide, harnessing the knowledge of scientists and the enthusiasm of citizens to implement conservation programs. Xerces works to raise awareness about the plight of invertebrates and to gain protection for the most vulnerable species before they decline to a level at which recovery is impossible.

The Honorable Deb Haaland
Secretary, U.S. Department of Interior
1849 C Street, NW Washington, DC 20240

Dear Secretary Haaland,

Pursuant to Section 4(b) of the Endangered Species Act ("ESA"), 16 U.S.C. § 1533(b); Section 553(e) of the Administrative Procedure Act, 5 U.S.C. § 553(e); and 50 C.F.R. § 424.14(a), the Xerces Society for Invertebrate Conservation hereby petitions the Secretary of the Interior, through the United States Fish and Wildlife Service ("FWS," "Service"), to protect the large marble butterfly (*Euchloe ausonides*) under the ESA as a Threatened Species. We additionally request that the type subspecies of the large marble butterfly, *Euchloe ausonides ausonides*, be listed as an Endangered Species.

The large marble butterfly occurs in the states of Alaska, California, Colorado, Idaho, Michigan, Minnesota, Montana, Nebraska, Nevada, New Mexico, North Dakota, Oregon, South Dakota, Utah, Washington, and Wyoming. Populations of *Euchloe ausonides* also occur across several Canadian provinces. This species has declined drastically in abundance across this broad range, including multiple documented extirpations and declines at almost every site with long-term monitoring data. The large marble's broad geographic range also exposes the species to numerous threats including widespread habits loss and degradation from multiple causes, introduced predators, exposure to pesticides, stress to individuals and food sources from effects of climate change, and inadequate pesticide regulations. The continued persistence of the type subspecies of the large marble butterfly, *Euchloe ausonides ausonides*, is of particularly grave concern as it has been extirpated at almost every long-term monitoring site within its range in lowland California and its overall range has been reduced by nearly one third.

FWS has jurisdiction over this petition. This petition sets in motion a specific process, placing definite response requirements on the Service. Specifically, the Service must issue an initial finding as to whether the petition "presents substantial scientific or commercial information indicating that the petitioned action may be warranted." 16 U.S.C. § 1533(b)(3)(A). FWS must make this initial finding "[t]o the maximum extent practicable, within 90 days after receiving the petition."

Submitted this 5th day of October, 2023.

Sincerely,

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Executive Summary

The large marble butterfly, *Euchloe ausonides*, is in steep decline and will face extinction without protection under the Endangered Species Act (ESA). *Euchloe ausonides'* historical range was widespread across much of western North America and parts of the Upper Midwest, with older (pre-2001) records in the United States including southern and eastern Alaska, California, Colorado, Idaho, Montana, western Nebraska, central and northern Nevada, northern New Mexico, western North Dakota, northern Minnesota, Oregon, western South Dakota, Utah, Washington, and Wyoming, and the Isle Royale National Park in Michigan. Though common historically, data from long-term monitoring sites across the western United States show extirpations from at least six sites and reduced abundances by 50% or more at eight other sites across the species' range. Recent studies using long term monitoring data have ranked the large marble as one of the western butterfly species most at risk of extinction in the next 50 years, with declines of this species surpassing other widespread butterflies including the monarch butterfly (*Danaus plexippus plexippus*), which has already met the criteria of a threatened species under the ESA. These data were used in population viability analyses, which estimated an almost 2/3 probability of extinction across all sites in the next 50 years. Additional analyses presented in this petition show that *E. ausonides* is observed less in recent time periods in 75% of its range, including in many regions with extensive habitat modifications from development and agriculture. The large marble butterfly and its habitat need protection across its entire range in the United States in order to prevent its extinction. As such, we request that the entire species be listed as Threatened under the ESA.

Of critical concern is the type subspecies of the large marble butterfly, *Euchloe ausonides ausonides*, which is already absent throughout much of its former distribution and is in danger of extinction in almost the entirety of its present range. Once present throughout each county in the Sacramento Valley and San Francisco Bay regions and common in both urban and rural locations, recent observations are almost entirely absent from the Sacramento Valley (in some places, not recorded since at least 2005), and remaining *E. a. ausonides* populations are relegated to beachfront areas along the San Francisco Bay and in the foothills of coastal ranges and the Sierra Nevada. Multiple long-term butterfly population monitoring sites have documented the disappearance of this butterfly subspecies while the occurrence of *E. a. ausonides* in museum and photographic records in multiple regions has plummeted by over 80%. Due to these extreme declines throughout the subspecies' entire range, we request that this taxon be listed as Endangered under the ESA.

The broad geographic distribution of the large marble butterfly has made it and its habitats vulnerable to numerous threats across its range. These threats include widespread and ongoing habitat loss and degradation from conversion to urban developments or agriculture, livestock grazing, removal of caterpillar food sources, and changes to the historical fire regimes. In addition, the large marble butterfly faces increased mortality from introduced predators and parasitoids, especially those used to control caterpillar pests of related Pieridae butterflies like the cabbage white (*Pieris rapae*). Exposure of the large marble butterfly to a varied and changing set of pesticides near urban areas, agriculture, or natural areas with economic value like grazing land or forests endangers populations and subpopulations across its range, not only near application areas but also in more distant locations where pesticides are found in non-target areas. Effects of climate change, especially increased temperatures and decreased precipitation, stress butterflies and their food sources at all life stages, further threatening the large marble across its entire range. These threats and their effects have decreased genetic diversity to an unknown extent, threatening the resilience of these populations in the face of rapid change and widespread imperilment. Existing regulatory mechanisms are not sufficient to protect this species from continued decline, and only one subspecies, the island marble (*E. ausonides insulanus*), consisting of a single extant population, is currently protected by the ESA.

As a widespread and historically common species, the large marble butterfly is an important part of western landscapes, serving as an herbivore of numerous plants and as a food source for an array of other invertebrates, reptiles, amphibians, birds, and mammals. Butterflies are a charismatic group of insects, well understood by biologists and often used as indicators of local ecological quality or regional insect population trends. Numerous extirpations and steep declines in abundance across this species' range are cause for concern, and the information compiled in this petition presents substantial evidence that *Euchloe ausonides* meets multiple criteria for inclusion as a Threatened Species, and that *Euchloe ausonides ausonides* meets multiple criteria for inclusion as an Endangered Species, under the U.S. Endangered Species Act.

Candidate Background, Status, and Listing History

The species-level taxon *Euchloe ausonides* has no legal protection under the Endangered Species Act or any state endangered species act. Because of its broad distribution and numerous documented occurrences, conservation assessments of the species and many of its widespread constituent subspecies do not reflect the threats from ongoing population declines described in this petition. The species-level taxon is listed as Globally Secure (G5, common, widespread, abundant, and lacking major threats or long-term concerns) and Nationally Secure within the United States (N5, at very low or no risk of extirpation in the jurisdiction due to a very extensive range, abundant populations or occurrences, with little to no concern from declines or threats) by the NatureServe organization (NatureServe 2022*a*). The species is listed either as Regionally Secure (S5) or Regionally Not Ranked (SNR) in most states within its range. However, it is listed as Regionally Critically Imperiled (S1, typically having 5 or fewer occurrences, or 1,000 or fewer individuals) in Nebraska. The species is considered Possibly Extirpated (SH, known from only historical records but still some hope of rediscovery) in Michigan, though contemporary observations of *E. ausonides* do occur in Michigan so this status is out of date. In Canada, the species is listed as Nationally Secure (N5), and is listed as Regionally Secure (S5) in Alberta and British Columbia; as Regionally Apparently Secure (S4, uncommon but not rare, but with some cause for long-term concern; typically having 101 or more occurrences, or 10,0001 or more individuals) in Manitoba, Northwest Territories, Saskatchewan, and Yukon Territory; and as Regionally Vulnerable (S3, rare; typically having 12 to 100 occurrences, or 3,001 to 10,000 individuals) in Ontario (NatureServe 2022*a*).

Of *Euchloe ausonides*' seven subspecies, only the island marble, *E. a. insulanus*, is listed by NatureServe as Critically Imperiled (very high risk of extinction or collapse due to very restricted range, very few populations or occurrences, very steep declines, very severe threats, or other factors) globally, nationally, and regionally in Washington (NatureServe 2022*b*). It is also listed as a Sensitive Species by the Washington Bureau of Land Management office and is a Candidate Species under the Washington Endangered Species Act (U.S. Department of Agriculture & U.S. Department of the Interior 2022; Washington Department of Fish and Wildlife 2023). All other subspecies in the United States lack any ranks by NatureServe, and none are listed as Sensitive Species by any U.S. Forest Service region or state Bureau of Land Management office. The subspecies *E. a. palaeoreios* (Johnson 1976) was listed as Regionally Critically Imperiled (S1) by the Nebraska Natural Heritage Program in 2015 and is considered a Tier 2 Species of Greatest Conservation Need by Nebraska Game and Parks; however, listing in this Tier has no legal or regulatory ramifications (Schneider et al. 2018). *Euchloe ausonides palaeoreios* and *E. a. transmontana* are not included in the NatureServe database at this time and *E. ausonides ogilvia* has not been assessed for the United States but is listed as Globally Secure (G5T5), Nationally Secure (N5) in Canada, Regionally Apparently Secure (S4) in the Yukon Territory, and Regionally Vulnerable (S3) in British Columbia (NatureServe 2022*c*).

The island marble, *Euchloe ausonides insulanus*, was first petitioned for listing as a Threatened or Endangered Species under the U.S. Endangered Species Act on December 11, 2002. It received a positive

90-day finding on February 13, 2006 (U.S. Fish and Wildlife Service 2006*a*), but after a review of scientific and commercial evidence, the Service concluded that listing was not warranted on November 14, 2006 (U.S. Fish and Wildlife Service 2006*b*). The subspecies was petitioned again to be listed as an Endangered Species on August 22, 2012, and the Service determined this petition provided substantial information indicating listed may be warranted on August 19, 2014 (Foltz-Jordan et al. 2012; U.S. Fish and Wildlife Service 2014). After reviewing the available scientific and commercial evidence, the Service found that listing of the island marble butterfly was warranted but was precluded by higher priority actions on April 5, 2016, and the taxon was added as a Candidate Species on December 2, 2016 (U.S. Fish and Wildlife Service 2016*a*, 2016*b*). On April 12, 2018, the Service proposed to list the island marble as Endangered and to designate approximately 812 acres of Critical Habitat, and the subspecies was officially listed as Endangered on May 5, 2020 (U.S. Fish and Wildlife Service 2018, 2020).

Natural History

Taxonomy

Euchloe ausonides is a member of the Pieridae family, containing butterflies known as the whites, marbles, and sulphurs. The species was first described by M.H. Lucas in 1852 from California as a part of the collection for Jean Baptiste Boisduval (Lucas 1852). The type specimen location was determined to be the San Francisco area in San Francisco County, California by Opler (1967). There are seven subspecies described according to the Pelham (2022*b*) North American catalog: the nominotypical *E. a. ausonides* (M.H. Lucas 1852), *E. a. coloradensis* (Hy. Edwards 1881), *E. a. mayi* (F. Chermock and R. Chermock 1940), *E. a. palaeoreios* (K. Johnson 1976), *E. a. transmontana* (Austin & J. Emmel 1998), *E. a. ogilvia* (Back 1991), and the federally Endangered island marble butterfly *E. a. insulanus* (C. Guppy & J. Shepard 2001). The nearest relative of *Euchloe ausonides* is *E. ausonia*, a Palearctic and southern European species. While some older taxonomic catalogs list *E. ausonides* as a subspecies of *E. ausonia* (Scott 1986), we follow the Pelham catalog and the Integrated Taxonomic Information System in this petition, which consider *E. ausonides* to be a valid species (ITIS 2022; Pelham 2022*b*). The nearest Nearctic relatives are *E. creusa*, *E. hyantis*, *E. lotta,* and *E. olympia* (Back et al. 2011).

Order: Lepidoptera
- Family: Pieridae (whites, marbles, and sulphurs)
  - Subfamily: Pierinae
    - Tribe: Anthocharidini
      - Genus: *Euchloe*
        - Species: *ausonides*
          - Subspecies: *ausonides*, *coloradensis*, *insulanus*, *mayi*, *ogilvia*, *palaeoreios*, *transmontana*

(ITIS 2022)

Description

Adults

Euchloe ausonides adults are medium sized butterflies, measuring between 1 ½ to 2 inches across both wings (Austin & Emmel 1998). Females are generally slightly larger than males. Ventral coloration between the sexes is generally similar, with a complex of green swirls with white spots ("marbling") across the entire hindwing. Veins tend to be yellower in shade compared to the surrounding green marbling (Fig. 1). The ventral forewing is mostly white with a narrow black cell-end bar in the middle and light black speckling along the leading forewing edge. Forewing margins have variable amounts of light green and/or black patterning and black checkering on the fringes.

Dorsal wing surfaces vary slightly between males and females. Males have white forewings with a black cell-end bar and black patterned margins. Hindwings have a light version of the marbling on a white background. Females are similarly patterned on the dorsal forewing but are typically a lighter shade of black. The hindwings of some females have a buff-yellow background as opposed to a white background (known as the *flavidalis* form, var. *flavidalis*, Scott 1986).

In the related *Euchloe hyantis* and *E. lotta*, the veins on the ventral hindwing are closer in shade to the surrounding green margins and the green marbling has a grayer shade compared to *E. ausonides*. There are no *flavidalis* form females in *E. hyantis* or *E. lotta*, and the shading on the dorsal hindwing is usually lighter compared to *E. ausonides*. The northern marble, *E. creusa*, tends to be smaller and the marbling tends to be denser compared to *E. ausonides*.

Subspecies Descriptions

A map of *Euchloe ausonides* occurrences, color coded by approximated subspecies distributions, is shown in Figure 2. Note that specimens were not identified to the subspecies level to create this map, but we have organized them as such following the ranges described in Pelham's catalog (Pelham 2022*b*) and some other regional field guides (Shapiro & Manolis 2007; Davenport 2018, 2020), which we follow throughout this petition; the classification of some Canadian and Pacific Northwest populations differs in other catalogs (Guppy & Shepard 2001).

Euchloe ausonides ausonides, originally described from San Francisco, is the largest subspecies with a forewing length averaging 0.95 inches (Austin & Emmel 1998). The neighboring *E. ausonides transmontana* is smaller, with an average forewing length of 0.88 inches, and forewing margins of *E. a. transmontana* are also narrower. Other morphological differences include less yellow in the ground color of the ventral side and broader and deeper green marbling in *E. a. transmontana*, sometimes obscuring the yellow veins. Finally, the leading forewing edges have less black speckling and there is a much lighter yellow hue on the dorsal hindwings (Austin & Emmel 1998).

Figure 1. Adult *Euchloe ausonides*, San Juan County, CO, 28 May 2022. Note the yellow veins compared to the green marbling. Also note the row of yellow scales behind the compound eyes, characteristic of the *coloradensis* subspecies. Photo credit: Nick Moore/iNaturalist, CC BY-NC 4.0.

Euchloe ausonides coloradensis is smaller than *E. ausonides transmontana* with an average forewing length of 0.80 inches (Johnson 1976; Austin & Emmel 1998). The distinctive dorsal forewing cell-end bar is smaller than other subspecies, and the forewing tip patterns are darker than *E. a. ausonides*. In addition, the ventral green marbling is broad and olive green, sometimes obscuring the typically yellow veins, and the green marbled patch that is furthest from the body on the ventral hindwing is typically separated from patches closer to the body on at least one side of the body (Fig. 1).

In the plains and prairies of northern Minnesota and Michigan, as well as in most lower Canadian populations, *Euchloe ausonides mayi* has fainter black markings on the ventral side on both the margins and in the cell-end bar compared to the nominate subspecies (Chermock & Chermock 1940).

The localized subspecies *Euchloe ausonides palaeoreios* is recognized not only by being restricted to old-growth conifer forests in the northwestern Great Plains but also by the marbling pattern on the lower (inner) ventral hindwing, resembling six smooth white patches along the edge of the wings. In addition, the black patterning on forewing tips is lighter than the neighboring *E. ausonides coloradensis* (Johnson 1976).

The northernmost subspecies, *Euchloe ausonides ogilvia*, has an average forewing length of 0.79 inches. As noted by Back (1991), the forewings are notably compressed in shape with dark black patterning on the dorsal forewing tips. Also noted in the description, dorsal hindwings have a light creamy yellowish hue to them, and the green marbling is less yellow and more olive green than in *E. ausonides ausonides*.

The most geographically restricted subspecies, *Euchloe ausonides insulanus,* is larger than the adjacent mainland *E. a. transmontana* subspecies. In addition, the green marbling on the ventral hindwings is broader than mainland subspecies, and there are yellow scales overlaying the marbling (Guppy & Shepard 2001). Third and fourth instar larvae also have markings near the spiracles that are not present in mainland *E. ausonides* subspecies (James & Nunnallee 2011; Lambert 2011).

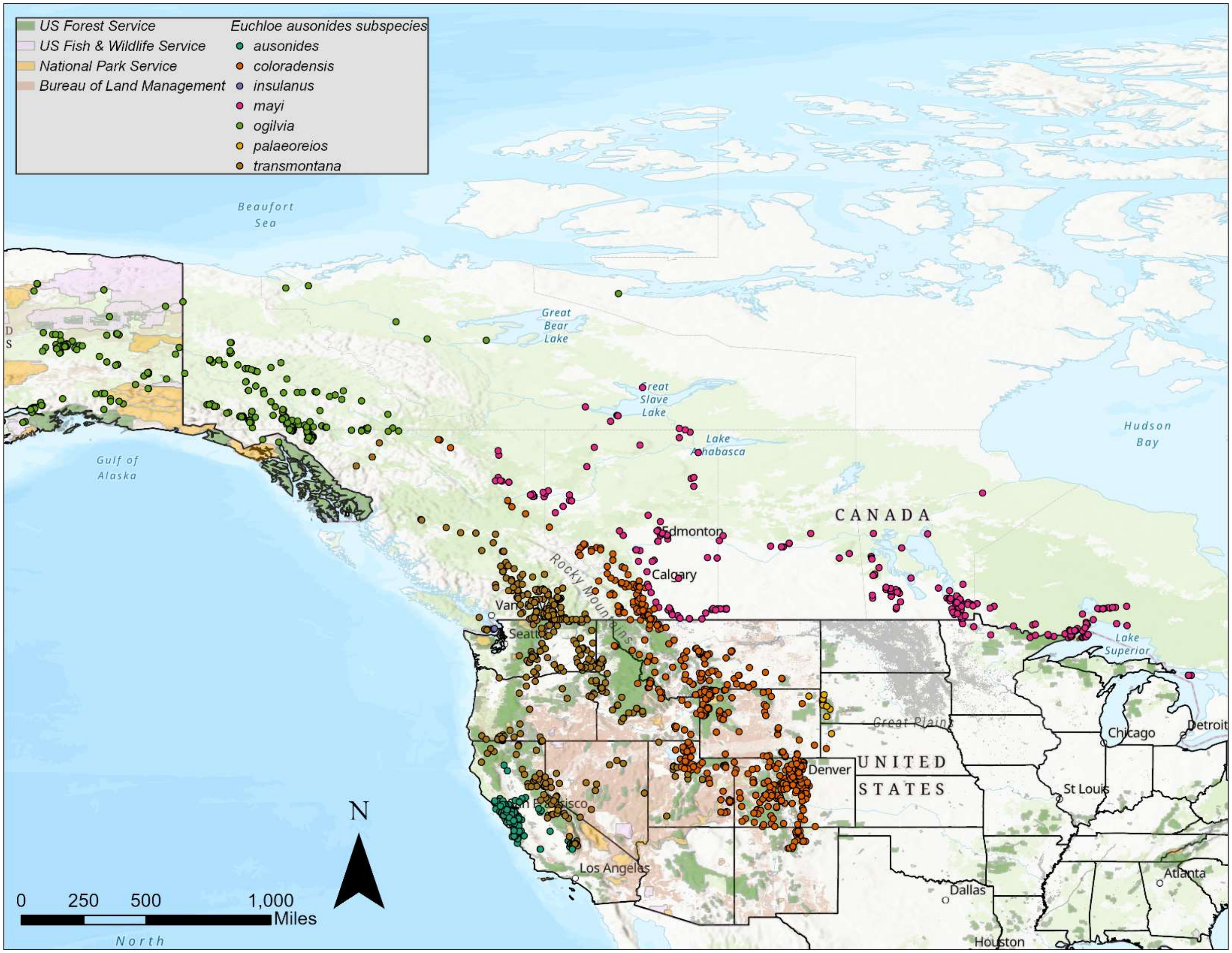

Figure 2. Distribution of *E. ausonides* across its range in the U.S. and Canada, color coded to illustrate approximated subspecies ranges. A full list of data sources for this map is listed in Appendix A. Note that specimens were not examined in the making of this map; subspecies assignment is based on subspecies placement during initial identification or subspecies boundary descriptions within literature.

Immature Stages

A full description of the immature stages is available in James and Nunnallee (2011) as well as in Lambert (2011), and comparative descriptions and drawings of *Euchloe* immature stages are in Opler (1974). Eggs are slightly under 1 mm in length and white, turning red as they age. First instar larvae have yellowish orange bodies and black head capsules with numerous black bullae (bumps) on the body. Second instar larvae are a dull yellow with light grayish purple stripes running along the top and sides, and the head is tan with brown spots. Later instars have a grayish-green color above and a green color below underneath the striping (Guppy & Shepard 2001). In the third instar, the yellow color along the body is brighter, the black bullae give the body a spotted appearance, and the head is a light brownish yellow with black spots (James & Nunnallee 2011). In the fourth instar, the yellow and purple striping along the body is brighter than ever, the bullae are again black and prominent, and the head is a dull greenish yellow with black spots. The fifth and final instar caterpillars are up to 35 mm in length, have bright purple, white, and green stripes along the body, black spotting from the bullae, and the head is gray with black spots. Small variations do occur between Alaskan and Canadian populations and the continental United States populations (Guppy & Shepard 2001).

Prepupal larvae turn a dark purple color (Opler 1974; Guppy & Shepard 2001; Scott 2020). The pupa has a thin overall shape and a light to medium brown color with gray or brown stripes along the dorsal and lateral surfaces (Guppy & Shepard 2001; James & Nunnallee 2011; Scott 2020).

Table 1. List of known caterpillar food plants for *Euchloe ausonides*. Relevant subspecies for each source are listed below.

Plant Species	Source
Arabis furcata	1, 3, 6
Arabis hirsuta	1, 3, 6
Barbarea orthoceras	1
Barbarea vulgaris	1
Boechera atrorubens	1,3
Boechera lyalli	1, 6
Boechera perennans	9
Boechera pinetorum	1, 5
Boechera retrofracta	1,3
Boechera sparsiflora	9
Boechera spatifolia	6
Boechera stricta	1, 6
Boechera suffrutescens	1, 6
Brassica napus	1, 6, 10
Brassica nigra	1, 10
Brassica rapa	1, 10
Caulanthus lasiophyllus	1, 6
Descurainia californica	1
Descurainia incana	1, 6
Descurainia incisa ssp. *incisa*	1
Descurainia pinnata ssp. *brachycarpa*	1, 6
Descurainia sp.	1, 2, 5, 6
Erysimum capitatum	1
Hirschfeldia incana	1
Isatis tinctoria	1, 2, 5, 9
Lepidium ramosissimum	4, 5
Lepidium virginicum ssp. *menziesii*	7
Raphanus sativus	4, 10
Sinapis arvensis	5
Sisymbrium altissimum	5, 9, 10
Sisymbrium linifolium	5
Sisymbrium loeseli	8
Sisymbrium officinale	5, 7
Turritis glabra	6

Sources:	Subspecies
1) Scott (1986)	All
2) James and Nunnallee (2011)	*transmontana*
3) Scott (2020)	*coloradensis*
4) Austin and Leary (2008)	*transmontana*
5) Warren (2005)	*transmontana*
6) Opler (1975)	All
7) Pyle and LaBar (2018)	*transmontana, insulanus*
8) Austin and Emmel (1998)	*transmontana*
9) Butterflies of America (2022)	*coloradensis*
10) Shapiro and Manolis (2007)	*ausonides*

Life Cycle and Behavior

Eggs are laid by *Euchloe ausonides* females singly on leaf buds, flowers, stems, and leaves of host plants in the mustard (Brassicaceae) family (Opler 1974; James & Nunnallee 2011). Eggs are almost always laid on the terminal flower buds, but Guppy and Shepard (2001) observed eggs on leaves in Canadian populations. As with some other Pieridae species, females sometimes prefer to lay only one egg per plant, but this is not always the case (Scott 1975; Shapiro 1984). The full list of documented larval food sources is listed in Table 1.

Eggs hatch in approximately four to five days (James & Nunnallee 2011). Larvae feed primarily on flowers and fruit, and may gather together vertically near flowers, possibly covered with loose silk (Opler 1974; Scott 1986, 2020). Larvae develop quickly, reaching pupation in slightly over two weeks (James & Nunnallee 2011). Larvae either pupate on host plant stems or “wander” some distance before attaching themselves to pupate on a stable overwintering surface like a small twig; *Euchloe ausonides insulanus* caterpillars move up to 4 meters to find a pupation site (Opler 1974; James & Nunnallee 2011; Lambert 2011; Scott 2020).

In most of its range, *E. ausonides* overwinters as a pupa and emerges as an adult between mid-April and June, or up to early July further north (Scott 1986, 2020; Warren 2005; Davenport et al. 2007; Shapiro & Manolis 2007; Davenport 2018). Pupae may be capable of diapausing more than one season in some locations (Scott 2020). In the Sacramento Valley, *E. ausonides ausonides* adults emerge between February and April, with a second brood between May and July that has now largely disappeared (Scott 1986; Shapiro & Manolis 2007). All other subspecies have a single brood.

Adults often nectar on flowers, with recorded nectar species of many colors; a full list of recorded nectar plants is in Table 2 but there are likely others. Adults may prefer yellow or white flowers, including those of its caterpillar food plants, and have also been noted visiting scat and mud (Scott 2020). Males often patrol linear features such as streams, valley bottoms, or berms in a straight-line flight (Shapiro & Manolis 2007). Adult males can glide a few meters at a time while searching for females, typically a few feet off the ground, and will rest with wings open or closed; males are capable of traveling several hundred meters or more a day (Scott 1975, 2020). Adults will spend overnight periods in grasses or woody shrubs (Scott 2020).

Adult lifespans across the species' range have not been measured, but lifespans of *Euchloe ausonides insulanus* are approximately 5 to 9 days (Lambert 2011). Individual fecundity of *E. ausonides* has not been measured but appears to depend on host plant phenology, host plant species, and spring weather conditions among other variables; females of the European species *Pieris napi* laid an average of between 90 and 140 eggs depending on whether butterflies displayed a spring or summer phenotype, and this range is likely similar for *E. ausonides* (Karlsson & Johansson 2008; Lambert 2011).

Table 2. List of documented nectar plants for *Euchloe ausonides*, along with regions where use of each plant species was documented. Relevant subspecies for each source are listed below.

Plant Species	Source
Achillea millefolium	4
Alcea rosea	4
Amsinckia sp.	1, 2
Arabis sp.	3
Arnica cordifolia	4
Barbarea orthoceras	3
Berberis repens	4
Boechera stricta	4
Brassica nigra	3, 4
Cardamine cordifolia	4
Carduus sp.	1
Cerastium arvense ssp. *strictum*	3, 4
Cirsium arvense	4
Cirsium sp.	1, 2
Dichelostemma capitatum ssp. *capitatum*	4
Draba stenoloba	4
Erodium cicutarium	4
Eruca vesicaria ssp. *sativa*	2
Erysimum capitatum	3, 4
Eschscholzia californica	4
Heterotheca villosa	4
Mertensia lanceolata	4
Packera cana	4
Packera fendleri	4
Physaria montana	4
Plantago lanceolata	3, 4
Potentilla pulcherrima	4
Prunus americana	4
Ranunculus sp.	4
Raphanus sativus	3, 4
Rubus sp. (blackberry)	4
Senecio crassulus	4
Senecio integerrimus	4
Silybum sp.	1
Sisyrinchium bellum	4
Taraxacum officinale	4
Thlaspi arvense	4
Townsendia hookeri	4
Trifolium pratense	4
Turritia glabra	4
Wyethia helenioides	4

Sources:	Subspecies
1) Shaprio and Manolis (2007)	*ausonides*
2) Pyle and LaBar (2018)	*insulanus, transmontana*
3) Scott (2020)	*coloradensis*
4) Scott (2014)	*coloradensis*

Population Structure

Euchloe ausonides tends to have a dispersed population structure due to the widespread nature of many of its preferred caterpillar food plants and its strong flight capability; adults are capable of flying an average of 400 meters a day, and up to over 1,400 meters daily in one study (Scott 1975, 2020) . Populations of the federally endangered *E. ausonides insulanus*, isolated on the San Juan Islands of Washington, are generally considered to have a metapopulation structure, limited by habitat structure and dispersal (Lambert 2011; Griesemer et al. 2021). For *E. ausonides insulanus*, occurrence records are clustered into "complexes" when they occur within 600 m, and occurrence complexes within 1.2 km of each other are grouped together (Griesemer et al. 2021).

Habitat

Larval host plants and *Euchloe ausonides* populations can occur in a wide variety of open habitats, including streamsides, berms, desert washes, beaches, canyons, sagebrush steppe, grasslands, meadows, montane slopes, open tundra, and weedy flats (Shapiro & Manolis 2007; James & Nunnallee 2011; Philip & Ferris 2016; Pyle & LaBar 2018; Scott 2020). In earlier years, *Euchloe ausonides* was also found in a variety of anthropogenic habitats, including parks, waste areas, and ditch or levee edges, especially in the Sacramento Valley and San Francisco Bay regions (Shapiro & Manolis 2007). Guppy and Shepard (2001) list *E. ausonides'* habitats in Canada as "meadows at all elevations east of the Coast Range, with occasional populations in the coastal mountains." Notes from various specimens and observations include habitat descriptions of chaparral, coastal prairie or coastal shrubland, grassy knolls, gravelly hilltops, hillside slopes and open hillsides, juniper and sage shrubland, meadows, mud banks, roadside clearings and road shoulders, and serpentine outcrops (SCAN 2021). A map of habitat suitability across *E. ausonides'* range using occurrence data, bioclimatic variables, and expert-derived range maps is shown in Figure 3.

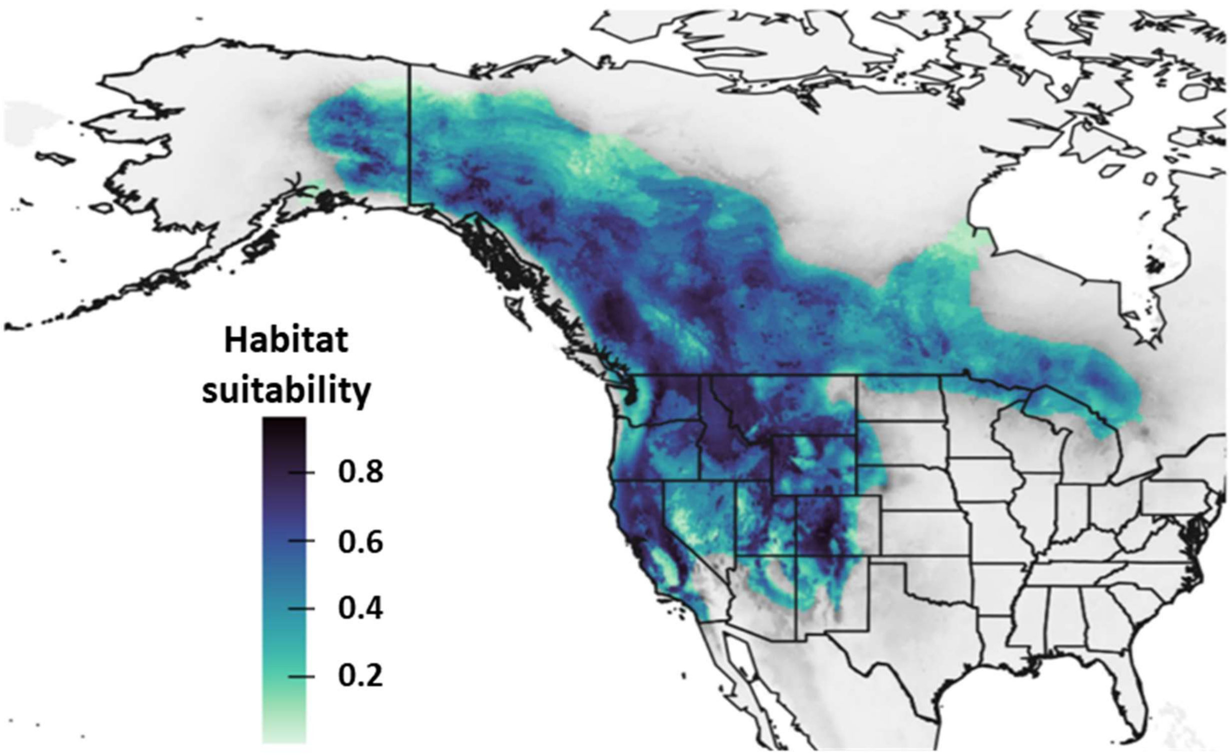

Figure 3. Habitat suitability for *E. ausonides* based on a MaxEnt model including elevation, 19 bioclimatic variables, the International Geosphere-Biosphere Programme land cover classification, and the geographic border of the range based on an integrative model that combines habitat suitability with expert-derived ranges. Darker colors indicate higher suitability. Source: Grames et al. (unpublished data).

Within these habitats, the Brassicaceae plants that are required for *Euchloe ausonides* caterpillar development favor disturbed environments for seed germination. These disturbances may include natural phenomena such as rodent burrows, treefalls, fires, or soil erosion from seasonal stream flow, or anthropogenic disturbances including roadside vegetation management or agricultural soil cultivation (Griesemer et al. 2021).

Griesemer et al. (2021) list the required habitat components for *Euchloe ausonides insulanus*, including low- to medium-density host plant populations for egg laying and larval development, nearby undisturbed standing vegetation for pupation sites, flowering plants for nectar and mating habitat, and a diverse topography including large open areas for mating, dispersal, and egg laying. Given that other subspecies of *E. ausonides* also appear to move short distances away from host plants to pupate and diapause (Scott 2020), the requirement of disturbance for host plant germination and less disturbed habitat for pupation suggests a mix of disturbance regimes is necessary for the success of all *E. ausonides* life stages.

Population Status and Distribution

Populations of *Euchloe ausonides* are declining across the species' range. The species is part of a western butterfly community declining in abundance by 1.6 % per year (Forister et al. 2021). The large marble has been extirpated at six sites with long-term monitoring of butterfly communities, and has declined by over 50% at eight other long-term monitoring sites across its range (Forister et al. 2023). Analyses using data from these locations suggest the large marble has almost a 2/3 chance of going extinct at all monitored sites in the next 50 years (Forister 2023). Public records databases also show that *E. ausonides* is observed less frequently since 2013 in over 75% of its range. Because of ongoing threats to butterfly populations and to this species' required food sources and habitats, abundances are expected to continue to decline across *E. ausonides*' range and the number of extirpations is likely to increase, putting this species at risk of becoming endangered in the foreseeable future. The observed extirpations at long-term monitoring sites, reduced abundances at other monitoring sites, and reduced numbers of recent observations--described in detail in the following sections--are all indicative of a species with many fewer individuals across the vast majority of its range.

Delaying protection for *Euchloe ausonides* under the Endangered Species Act threatens this species with extinction. Though *E. ausonides* is geographically widespread and has historically been common in many locations (Warren 2005; Shapiro & Manolis 2007; Scott 2020), even common species can become endangered if abundances decline rapidly without protection, such as the now extinct passenger pigeon (*Ectopistes simigratorius*) and Rocky Mountain grasshopper (*Melanoplus spretus;* (Chapco & Litzenberger 2004; Gaston & Fuller 2008; Hung et al. 2014). In fact, *E. ausonides'* distribution exposes it to a larger number of threats able to degrade or destroy habitats or contribute to increased mortality across its range (Lindenmayer et al. 2011). Reduced range sizes are generally associated with reduced abundances across species, and abundances are typically highest in the core of a species' range, so reduced abundances in the core of this species' range in western grasslands may be particularly influential to the species' overall abundance (Brown 1984). Delays in protection for *E. ausonides* and assumptions about its resiliency without timely intervention will quickly lead to it being endangered in the foreseeable future.

Historical Distribution

Euchloe ausonides' historical range was widespread across much of western North America and parts of the Upper Midwest, with older (pre-2001) records in the United States including southern and eastern Alaska, California, Colorado, Idaho, Montana, western Nebraska, central and northern Nevada, northern New Mexico, western North Dakota, northern Minnesota, Oregon, western South Dakota, Utah, Washington, and Wyoming, and the Isle Royale National Park in Michigan; see Figures 4 a-c for maps of occurrences focusing on the United States (Brock & Kaufman 2006 p. 54; SCAN 2021; GBIF 2022a; Lotts & Naberhaus 2022). Historical records in Canada include most of the Yukon Territory and British Columbia east of the Coast Range, the western Northwest Territories, western Nunavut, Alberta, southern Saskatchewan, southern Manitoba, and southern Ontario (Guppy & Shepard 2001; GBIF 2022a). Similar to populations in western Canada, populations in Washington and Oregon are all east of the crest of the Cascade Mountains but extend west further south in the Siskiyou Mountains of southwest Oregon. In

California, Garth and Tilden (1986) describe *E. ausonides* as the most common *Euchloe* species in low elevations in central and northern areas of the state, and Shapiro and Manolis (2007) note that previous to 2005 the species was found in all counties in the Sacramento Valley and San Francisco Bay regions. Notably, the Endangered subspecies *E. ausonides insulanus* was historically found on Vancouver Island and Gabriola Island, British Columbia, Canada, but was last seen in Canada in 1910 and was declared extinct by the Canadian government in 1999 (COSEWIC 2000). Populations were discovered in 1998 on San Juan Island and subsequently on Lopez Island (now extirpated) in San Juan County, Washington, with a total of five distinct populations on these two islands, though only one population is currently extant (Griesemer et al. 2021).

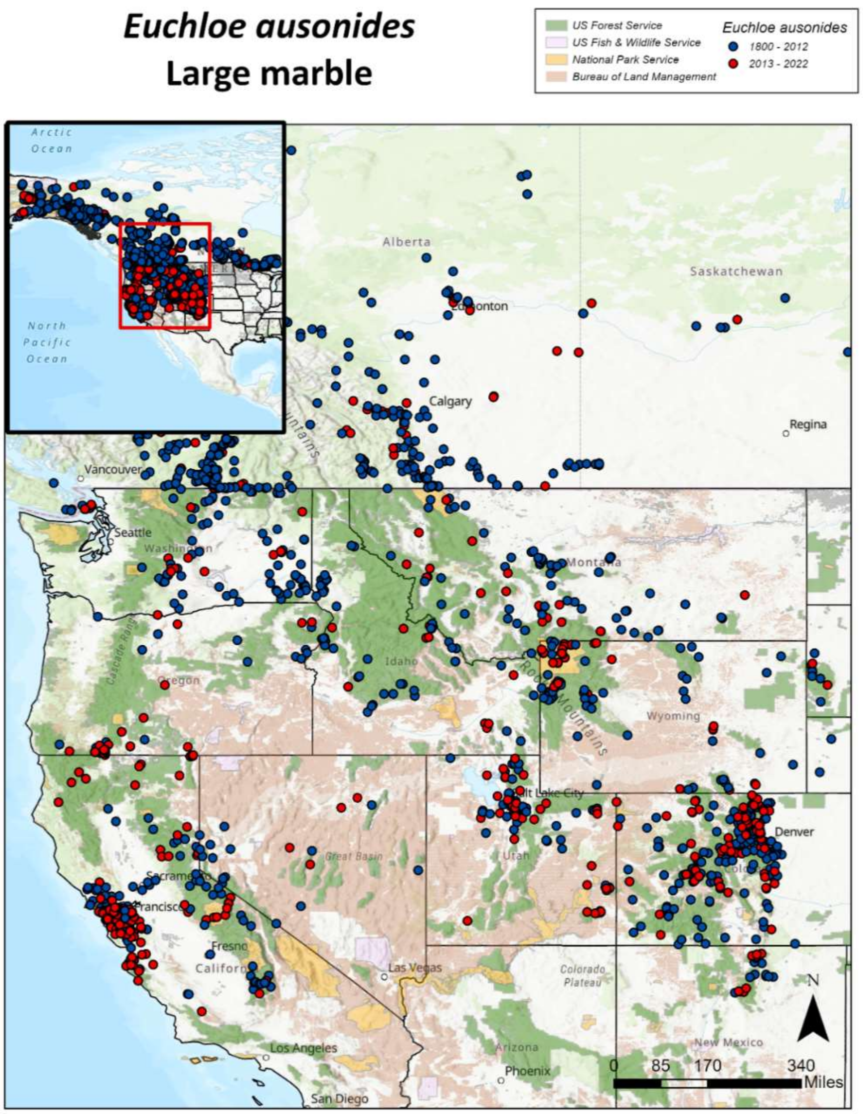

Figure 4a. *Euchloe ausonides* occurrences in the western continental United States. See data and map sources in Appendix A; note that some databases were not queried for Canadian observations. Maps have been split to allow for higher resolution viewing; see inset maps in each for geographical context.

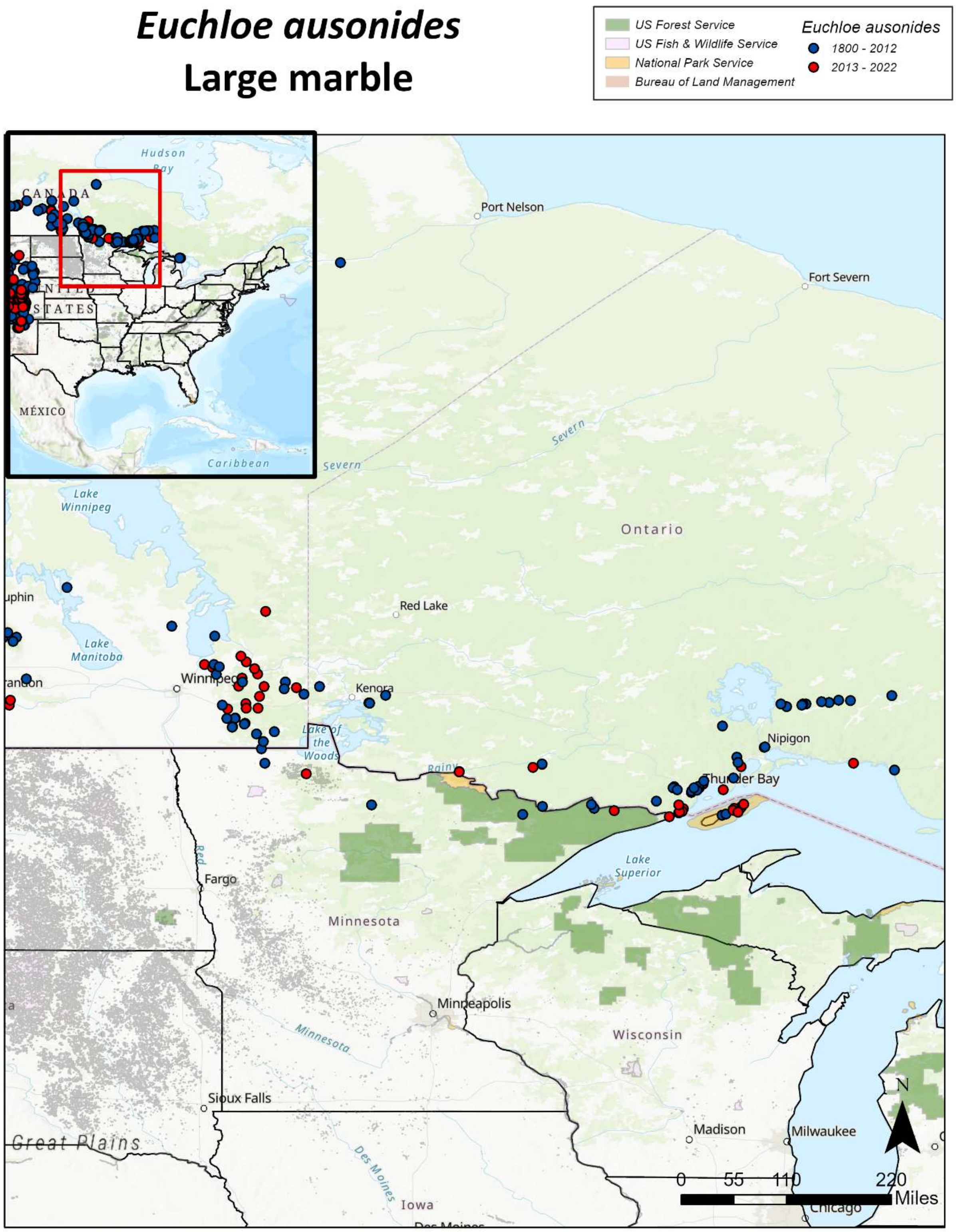

Figure 4b. *Euchloe ausonides* occurrences in the upper midwestern United States. See data and map sources in Appendix A; note that some databases were not queried for Canadian observations. Maps have been split to allow for higher resolution viewing; see inset maps in each for geographical context.

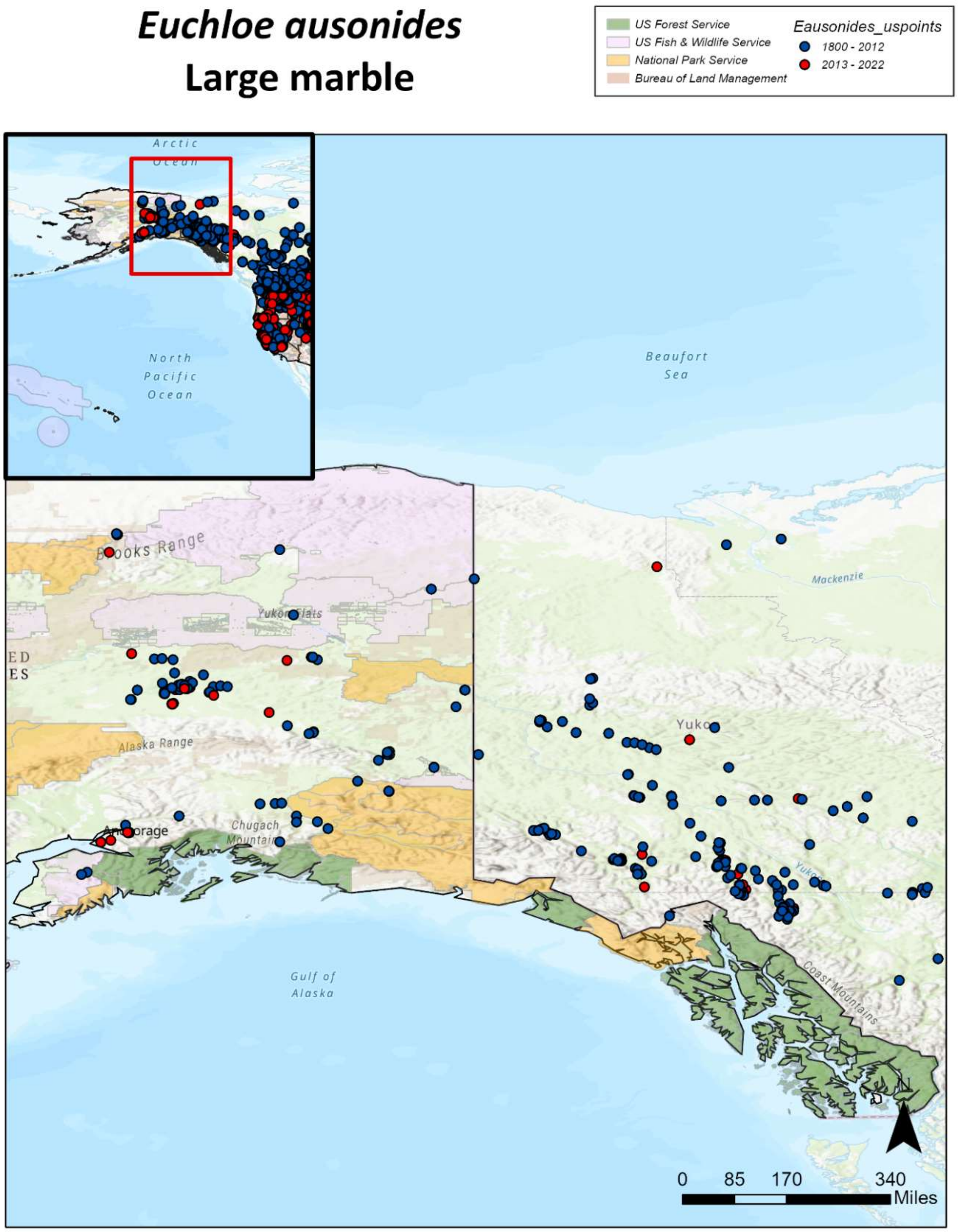

Figure 4c. *Euchloe ausonides* occurrences in Alaska. See data and map sources in Appendix A; note that some databases were not queried for Canadian observations. Maps have been split to allow for higher resolution viewing; see inset maps in each for geographical context.

Current Distribution and Population Status

The current distribution of *Euchloe ausonides* includes large sections of the species' historical extent but with many documented extirpations, varying by subspecies, and reduced abundances across much of the species' range. Recent studies by Forister et al. (2021, 2023) indicate that *E. ausonides* is declining in abundance at most sites where the species has been monitored since the 1980s (see map and cross-section of long-term butterfly monitoring locations that include *E. ausonides* used in these analyses in Figure 5). Forister et al. (2021) found declines in abundance of most of the 272 widespread western butterfly species, including *Euchloe ausonides* (Fig. 6). Using Bayesian Poisson regressions to model trends in long term butterfly monitoring data for all species studied across the western United States, Forister et al. (2021) estimated a continual 1.6% decline in total butterfly abundance per year, which translates to a 25% decline in overall butterfly abundance every twenty years.

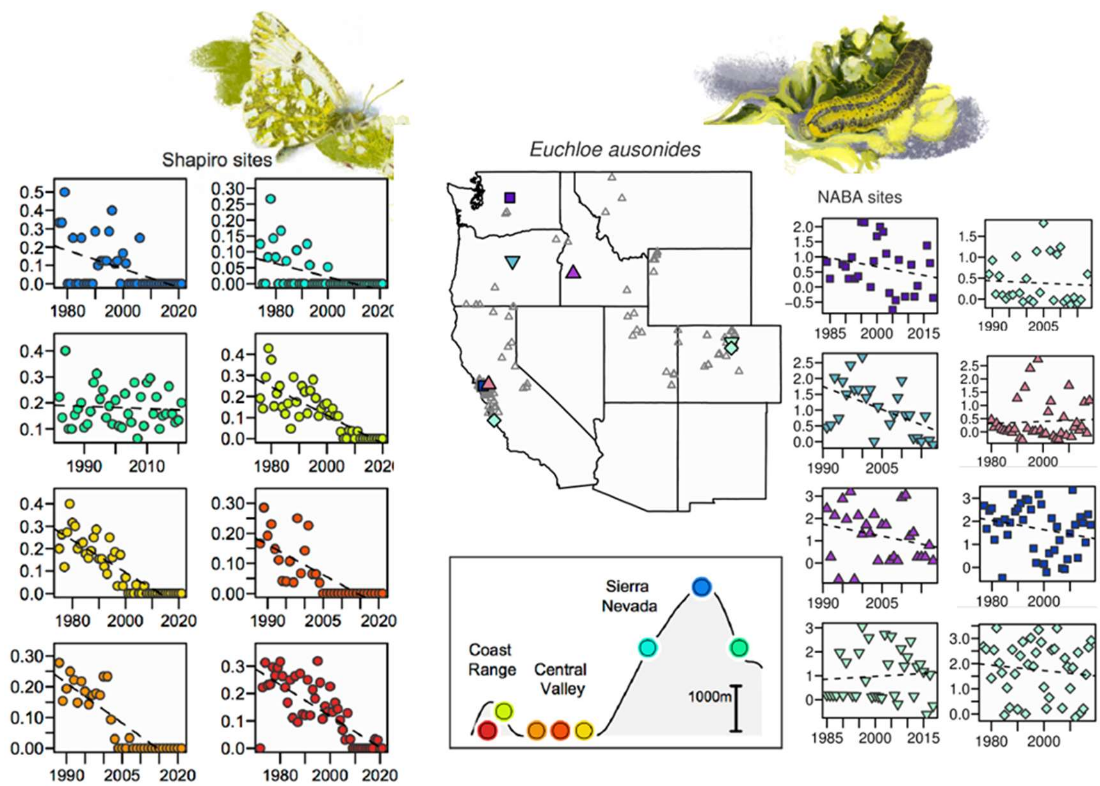

Figure 5. Examples of long-term monitoring data for *Euchloe ausonides* populations in the western United States. Data from 10 sites in California (shown in elevational cross-section of central California, bottom middle, and in detail in panels on the left; each site color-coded) were collected primarily by Art Shapiro, with contributions in recent years from the Forister lab. Data from annual North American Butterfly Association counts, shown in panels on the right, were collected from sites shown as corresponding colored shapes on the map (top middle). Gray triangles on map are iNaturalist observations (included here to provide distributional context and used elsewhere in related analyses). Figure from Forister et al (2023). Butterfly illustrations used with permission, Camryn Maher, copyright 2022.

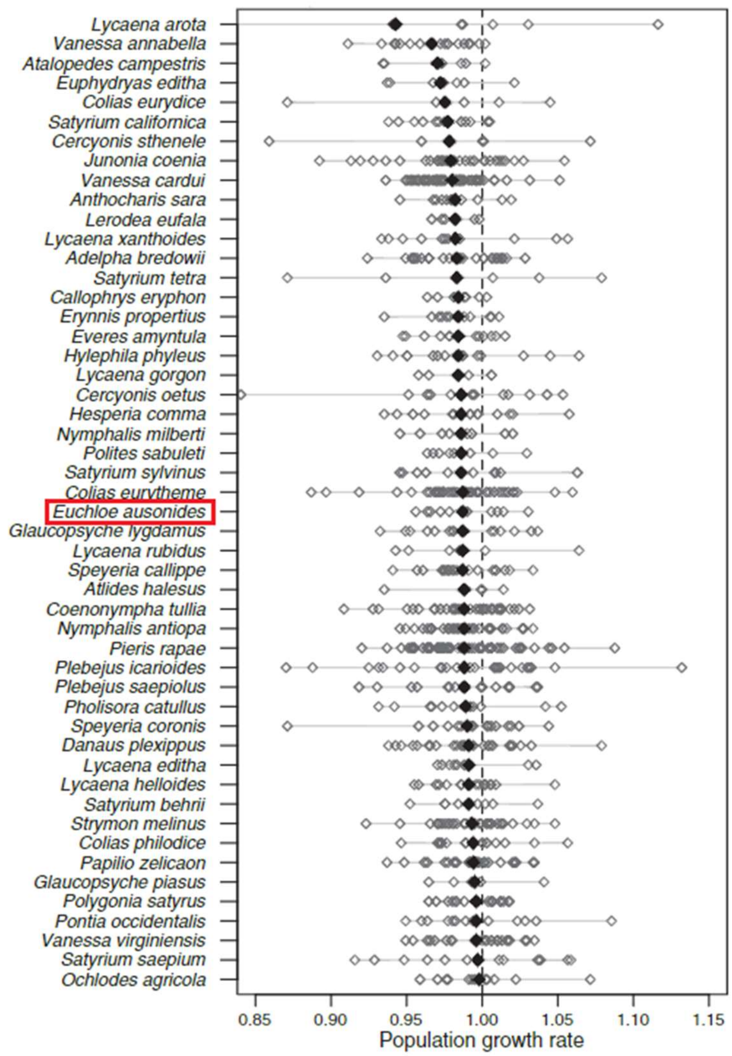

Figure 6. Population growth rates for a subset of western butterfly species analyzed in Forister et al. (2021) and shown in Figure 2 therein, with *Euchloe ausonides* highlighted here. Black diamonds are average growth rates across all North American Butterfly Association Annual Butterfly Count locations with that species present, and individual sites shown as open gray diamonds. Data points to the left of a growth rate equal to 1 are in decline.

While Forister et al. (2021) primarily investigated changes in abundance of regional butterfly communities over time, recent work using these same data highlight drastic declines in *E. ausonides* populations across its range relative to other western butterfly species. Both Forister et al. (2021) and (2023) use data collected by Art Shapiro (University of California, Davis) and the North American Butterfly Association's (NABA's) Annual Butterfly Counts. For the Shapiro monitoring program, presence/absence data are collected at 10 sites across Central California every two weeks throughout the local breeding season. The Shapiro monitoring program has been running for more than 50 years in Central California, providing a unique view into butterfly population trends in this region; this type of intensive, long-term butterfly monitoring does not exist elsewhere. NABA's annual butterfly counts are typically once-a-year inventories conducted around the same time each year on or near the height of the local butterfly season, and have been running for several decades at many sites. During these counts, participants survey sites within a 15-mile diameter of an established point, counting and identifying every individual butterfly encountered to the lowest possible taxonomic unit. Considering the unique limitations of each program's protocols, Forister et al. (2023) used population viability analyses and Bayesian binomial regressions to analyze the Shapiro and NABA datasets, respectively.

Analyses used for the NABA sites in Forister et al. (2023) consisted of hierarchical Bayesian linear Poisson regressions that combine historical data from all species at a given site to model changes in a given species' population size as a function of the year, site, and a species-specific growth rate. This growth rate was then used in Monte Carlo simulations to project the population size at these sites 50 years into the future. A species was considered 'extirpated' at a site when the projected population size dropped below 0.1 individuals at a site, and the results of these simulations were used to make species-level estimates of per-population extirpation, roughly equivalent to a probability of extinction. These simulations predict a 62.5% (lower bound= 25%, upper bound= 87.5%) probability of extinction of *Euchloe ausonides* in the next 50 years (Forister 2023).

Bayesian binomial regressions of the Shapiro dataset were combined with the results of these population extirpation estimates to form a quantitative rank, with error margins, for 184 widespread butterfly species for which long term monitoring data are available. Based on this ranking, which includes data from monitoring sites in California, Colorado, Idaho, Oregon, and Washington, *Euchloe ausonides* is one of the top five species most at-risk of extinction across its range in the next 50 years (Fig. 7); overlapping confidence intervals suggest these top species are all similarly imperiled. Note that the monarch butterfly (*Danaus plexippus*), which the FWS has determined warrants listing as Threatened under the ESA, is ranked number 30 in this analysis, whereas *E. ausonides* is ranked number 4; this analysis indicates that it is more imperiled than the monarch.

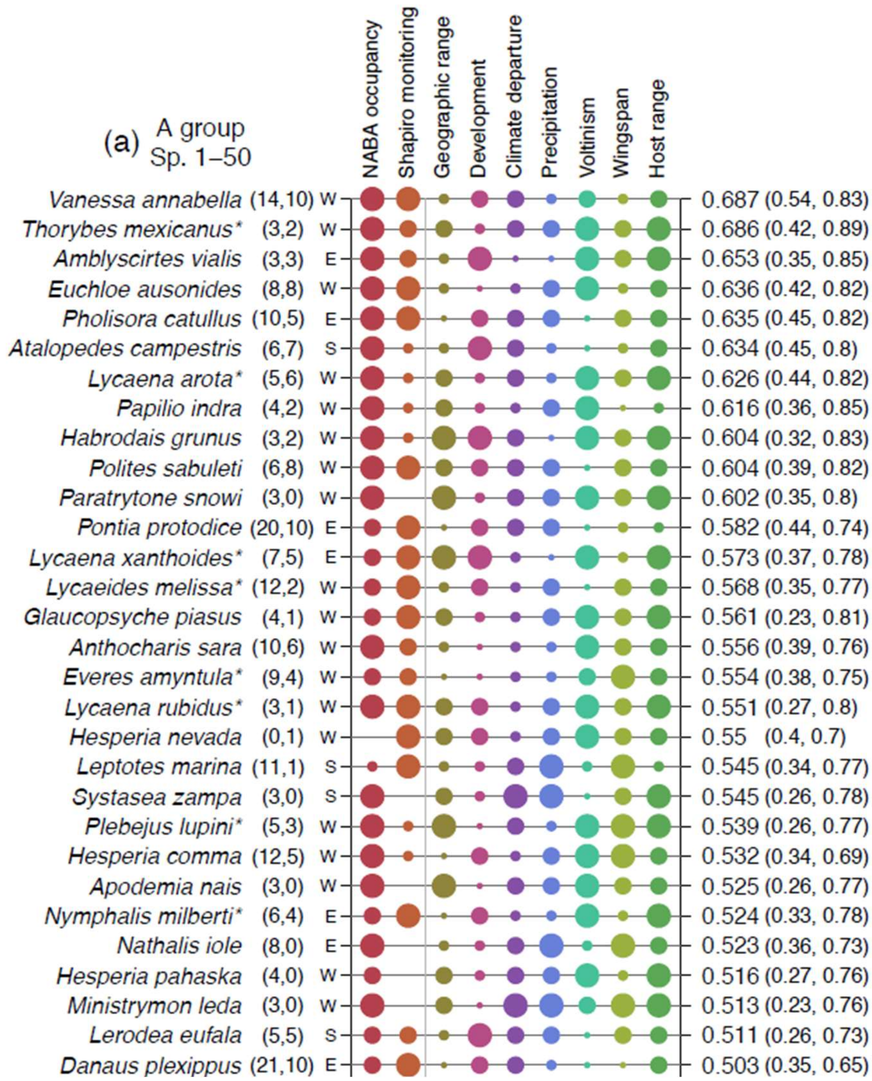

Figure 7. Quantitative risk ranking of widespread western butterfly species based on long-term monitoring data ("A" group in manuscript); full methodology in Forister et al. (2023). Average values of risk are followed by 85% credible intervals in parentheses. The overall risk ranking for the A group species is based solely on the first two variables (NABA occupancy and Shapiro monitoring, to the left of the vertical gray line). Bubbles and their sizes show the extent to which different variables are associated with higher (larger) or lower (smaller) risk for each species: a large circle under NABA occupancy, for example, marks a species that the authors infer as being at risk because of low forecast occupancy (probability of population persistence) across currently-extant locations. A secondary analysis, depicted to the right of the vertical gray line, which was not used in the quantitative risk ranking, approximates the exposure of species to human disturbances or potential risk due to biological traits: a large circle under development indicates a species at suspected higher risk of extinction because of high exposure to developed lands, and a large circle under geographic range indicates corresponding risk associated with a relatively small range. These variables are included here to represent possible risk factors associated with the observed declines. In this analysis, *E. ausonides* has a high risk due to its mostly univoltine phenology, changes in precipitation patterns, a limited number of plant genera used as larval hosts.

A separate analysis conducted for this petition using species occurrence data from the Global Biodiversity Information Facility (GBIF) database (GBIF 2022*a*, 2022*b*) further highlights the greatly reduced abundance of *Euchloe ausonides* across a significant portion of its range when comparing historical (pre-2013) and contemporary (2013 to 2022) records (Grames and Forister 2022, unpublished analysis; full method details in Figure 8). The results of this analysis show that *E. ausonides* is now observed less frequently than it was historically in 75% of its range where observations are available, including large sections of every subspecies' distribution and including regions of high habitat suitability (Fig. 2 and detailed in the Natural History: Habitat section). Data incorporated into this analysis for each cell are listed in Appendix B. Details of changes in occurrence, reported number of observations over time, and abundance are described for each subspecies in the following sections.

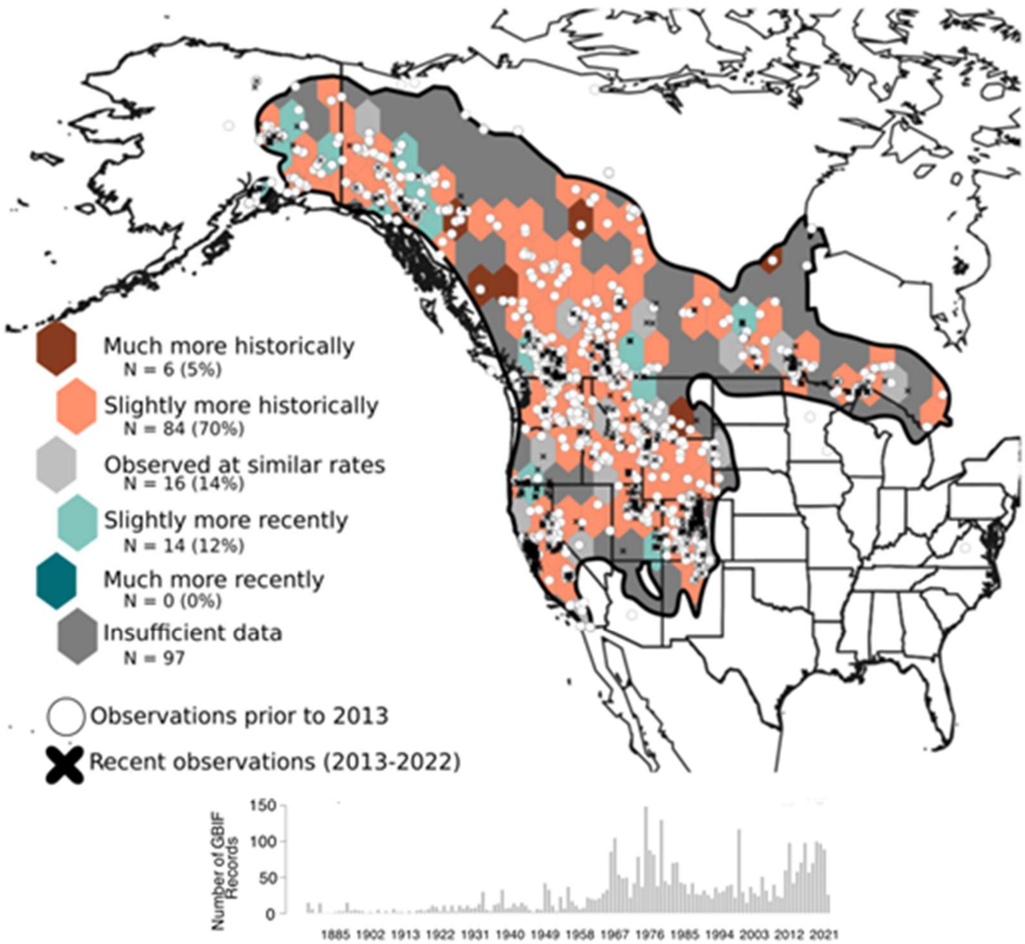

Figure 8. Observations from the Global Biodiversity Information Facility (GBIF) for *Euchloe ausonides*, with observations from the last ten years (2013 - 2022) as black "x" points and observations prior to that as white circles. The black border is the edge of the expert-derived species range from Glassberg (2017). Hexagons are colored based on whether effort-standardized *E. ausonides* records appear more frequently (green) or less frequently (red) in the last ten years. In this analysis, *E. ausonides* records for historical (pre-2013) and contemporary time periods (2013-2022) were search effort-standardized by dividing *E. ausonides* records by all GBIF butterfly records for a given area (colored hexagons) in the same time period. The historical, effort standardized value was then subtracted from the current, effort standardized value to characterize change through time, with values greater than five per 1000 observations (> 0.005) labeled as representing a location where *E. ausonides* was observed much more in the historical time period compared to the current time period; values greater than 1 per 1000 observations represent slightly more historical representation; and values less than 1 per 1000 observations represent no change. When *E. ausonides* had a greater frequency of historical observations compared to contemporary observations, values were parsed with the same thresholds as historical change (Grames and Forister 2022, unpublished analysis). Because sampling effort is standardized *within* cells and *within* each time period, results shown in Figure 7 report changes in the relative occurrence between eras in raw percentages. In well-sampled regions these values may be used to infer changes in *E. ausonides* relative abundance within cells between time periods. Grames and Forister, unpublished analysis. Bottom panel shows the frequency of *E. ausonides* records over time in the GBIF database.

Euchloe ausonides ausonides

The nominate subspecies of *Euchloe ausonides* had a historical distribution including largely low elevation portions of central western California, especially the San Francisco Bay region, extending south into San Luis Obispo and Tulare Counties, and extending east into the lower elevation foothills of the western Sierra Nevada Mountains from Placer to Yolo Counties (Fig. 2) (Garth & Tilden 1986; Davenport 2007; Shapiro & Manolis 2007; Davenport 2018, 2020; Butterflies of America 2022).

Euchloe ausonides ausonides has been extirpated from every long-term monitoring site in the Shapiro dataset, including Suisun Marsh and Gates Canyon in Solano County, as well as West Sacramento, North Sacramento, and Rancho Cordova in Sacramento County (Forister et al. 2022, Fig 5). Shapiro and Manolis (2007) note that this taxon is largely absent from the southern Sacramento Valley area since at least 2005, though populations persist in the San Francisco Bay near shoreline parks, preserves and marinas in Alameda County, California. Outside of the San Francisco Bay and Sacramento Valley, populations monitored during the NABA Annual Butterfly Counts near the southwest edge of this subspecies' range in California are in slight decline (Forister et al. 2022; Fig. 5). A separate Bay Area population monitored by NABA has also declined over 50% since monitoring began there, while another is stable but *E. ausonides* abundances are consistently low (Fig. 5). Perhaps most interestingly, *E. a. ausonides* was the only subspecies with two flight generations per year, with the first between February and April and a second brood between May and July; Shapiro and Manolis (2007) note that the second brood has almost entirely disappeared.

These transect analyses support the inferences of the comparisons of historical and recent records in this region, which show fewer recent observations across its entire range (Fig. 7). Locations that are particularly well sampled include the San Francisco Bay region with over 70,000 GBIF butterfly records, where recent records of *E. ausonides* are over 80% less common compared to historical records (Appendix B). Similarly, *E. ausonides* records are 66% less common in the central coast region and over 80% less common in the far southeastern extent of its range.

A separate Extent of Occurrence (EOO) analysis was also completed for *Euchloe ausonides ausonides* for this petition. Records of *Euchloe ausonides* from sources in Appendix B were assigned to this subspecies using either the specimen determination in the record or by using the geographic ranges and record descriptions in regional guides (Shapiro & Manolis 2007; Davenport 2018, 2020). Records were divided into a historical period (pre-2013, n= 319) and contemporary periods (2013-2022, n= 302). The EOO for each subset of observations was run using the IUCN EOO Calculator V 1.5 on ArcGIS Pro 3.0.2 (International Union for Concerned Scientists 2021). This analysis confirms the absence of *E. a. ausonides* across the majority of the Sacramento Valley and suggests the contemporary range of this subspecies has been reduced by over 30% compared to its historical range (pre-2013 range= 94,620 km^2, 2013-2022 range= 65,739 km^2; Fig. 9).

The combination of numerous documented extirpations, the loss of an entire flight generation, a reduction of almost 1/3 of its range, and steep reductions in relative abundance based on collected records across its entire distribution all suggest that *Euchloe ausonides ausonides* is at risk of extinction in the foreseeable future. Give that the threats discussed in the Current and Potential Threats section are likely to continue across its distribution, this subspecies is in need of protection as an Endangered Species to prevent extinction across its entire range.

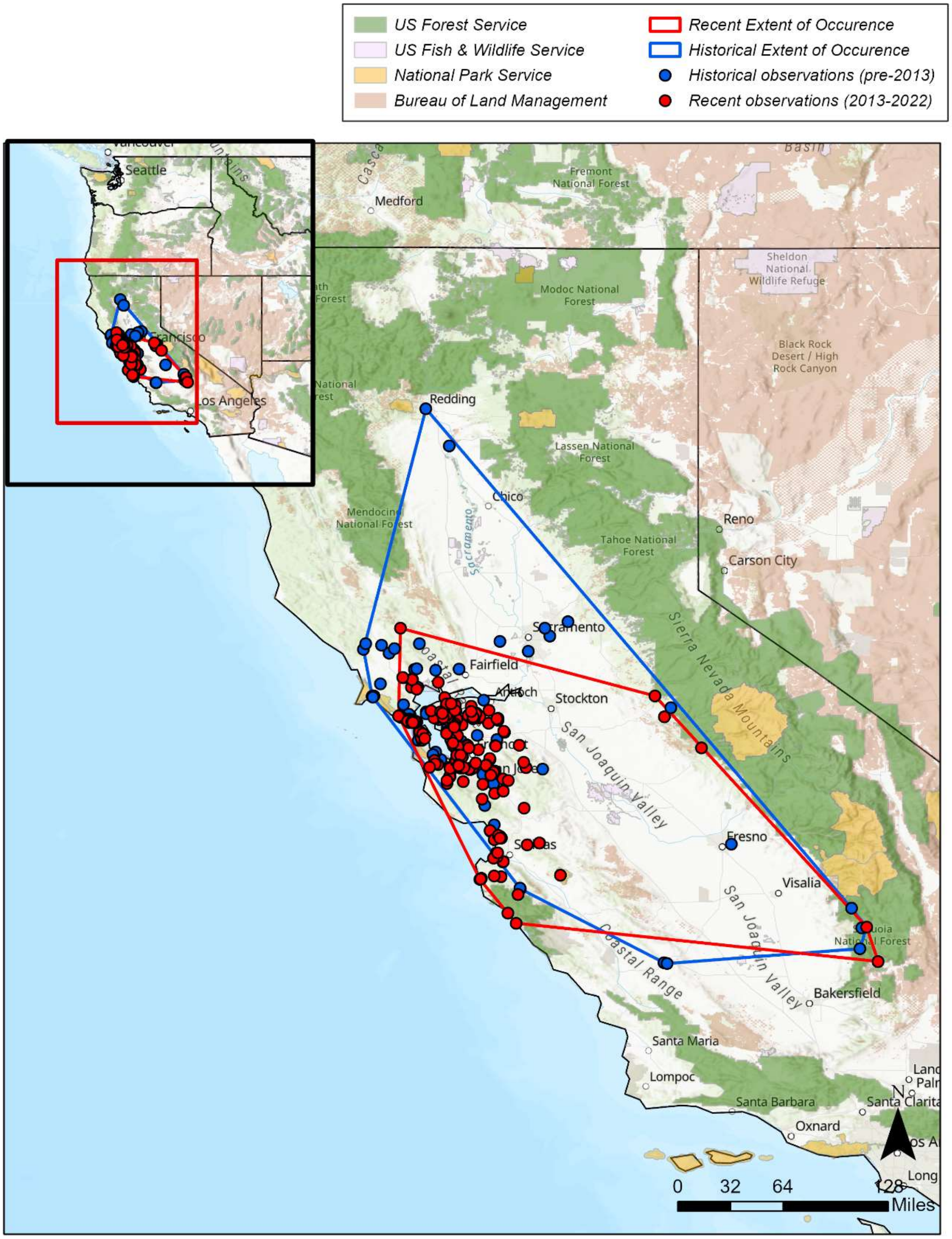

Figure 9. Extent of Occurrence (EOO) analysis for *Euchloe ausonides ausonides*. Blue dots and the blue outline are occurrences and the EOO from previous to 2013, and red dots and red outline are occurrences and EOO from 2013-2022. Note that recent sampling effort has increased substantially in some portions of the subspecies' range. There are 8,689 GBIF butterfly records for this region before 2013 and 61,622 butterfly records from 2013 to present; between these time periods the fraction of *E. ausonides* records in this same area decreased from 1.6% to 0.3% (see Appendix B for details).

Euchloe ausonides coloradensis
East of *Euchloe ausonides transmontana*, *E. ausonides coloradensis*' distribution forms the southeast extent of the species' range and includes eastern Idaho and eastern Utah, extending north to central Wyoming and Montana, south to New Mexico, and east towards eastern Colorado (Edwards 1881; Butterflies of America 2022). Some authors consider northern Montana populations to be *E. a. mayi*, and the exact boundary between these two subspecies is unclear in this region (Guppy & Shepard 2001). However, we follow the Pelham catalog of North American butterflies, which we consider to be the taxonomic authority for butterflies, and this source considers northern Montana populations to be part of *E. a. coloradensis* (Pelham 2022b, 2022a).

This subspecies occurs at two NABA long-term monitoring locations; in one, its population appears to be in slight decline, while at the other site nearby, it appears stable (Forister et al. 2022, Fig. 5). Comparisons of the frequencies of *E. ausonides* GBIF records within this subspecies' distribution show the species is recorded less frequently since 2013 across almost the entirety of its range from northern New Mexico across Colorado, Wyoming, eastern Utah and Idaho, Montana, and into the higher elevation regions of Alberta and British Columbia in Canada (Fig. 7). In well-sampled areas like north-central Colorado, the relative frequency of *E. ausonides* records since 2013 is almost half of its relative occurrence in that area in the past. Northern New Mexico is similar: with almost 10,000 GBIF butterfly records, *E. ausonides* records are almost 90% less frequent since 2013.

Euchloe ausonides insulanus
This subspecies historically occupied multiple islands in the Southern Gulf Islands archipelago of the U.S. and Canada, including Gabriola Island, San Juan Island, and Lopez Island. The last island marble in Canada was seen on Gabriola Island in 1908 (Griesemer et al. 2021). The Federally Endangered *Euchloe ausonides insulanus* currently has only one extant population, at American Camp on San Juan Island; the other populations have been extirpated since roughly 2006 (Foltz-Jordan et al. 2012; Griesemer et al. 2021). The remaining population has had extensive monitoring and that population is estimated to have between 300 and 500 individuals (Griesemer et al. 2021).

Euchloe ausonides mayi
This subspecies' distribution includes most of the lower Canadian populations, ranging from British Columbia east of the Coast Range and across Alberta, southern Saskatchewan, Manitoba, and southern Ontario. This subspecies is found primarily in prairie or taiga habitat (Chermock & Chermock 1940). Guppy and Shepard (2001) include populations as *E. a. mayi* as far south as central Oregon and Montana populations However, we follow the Pelham catalog of North American butterflies, which we consider to be the taxonomic authority for butterflies, and this source considers Pacific Northwest, northern Idaho, and northern Montana populations to be part of *E. a. coloradensis* (Pelham 2022b, 2022a). Using this definition, *Euchloe ausonides mayi's* distribution in the United States includes the northern counties of Minnesota, with observations from Roseau, Lake of the Woods, Koochiching, St. Louis, and Cook Counties. It has also been observed on Isle Royale National Park in Keweenaw County, Michigan.

Records of *E. ausonides* are sparse across much of *E. ausonides mayi*'s distribution, especially Saskatchewan and Manitoba and also including this subspecies' range in the United States in Minnesota and Michigan (Fig. 8). Where records are available, such as northwest Minnesota, *E. ausonides* records since 2013 are almost 75% less frequent compared to records prior to 2013 (Appendix B).

Euchloe ausonides ogilvia
This distribution of *Euchloe ausonides ogilvia* includes the species' northern extent in the tundra, plateau, and mountains of southern and eastern Alaska, Yukon Territory, northwestern British Columbia, Northwest Territories, and one observation from Nunavut (Back 1991; Guppy & Shepard 2001; Philip & Ferris 2016). United States observations of this subspecies in Alaska occur from the North Slope

southward and are found in Anchorage, Fairbanks North Star, Haines, Kenai Peninsula, Matanuska-Susitna, North Slope, Southeast Fairbanks, Valdez-Cordova, and Yukon-Koyukuk Counties (Philip & Ferris 2016; SCAN 2021; GBIF 2022a; Lotts & Naberhaus 2022).

Comparisons of the relative frequency of *E. ausonides* records in *E. a. ogilvia*'s range show recent records are less frequent across over half of its range in eastern Alaska, including the Fairbanks region, much of the border of eastern Alaska and into western Yukon Territory, and in the southeast along the coast with the Gulf of Alaska (Fig. 8). In the region with the best butterfly sampling near Fairbanks, with over 16,000 GBIF butterfly records, the relative frequency of *E. ausonides* records has decreased by 57% (Appendix B).

Euchloe ausonides palaeoreios

This subspecies is endemic to upland climax conifer forests in the far northwestern Great Plains region, including the Little Missouri River escarpments in western North Dakota, the Black Hills of far eastern Wyoming and western South Dakota, and the Pine Ridge of western Nebraska (Johnson 1976). Johnson notes in this article that "possible" occurrences include the Lone Pine Hills (possibly also called Long Pine Hills) of southeastern Montana and western South Dakota, and the Killdeer Mountains in western North Dakota (Johnson 1976), though no observations with locality information appear to exist in these locations. Johnson (1976) also notes that there were no "recent" observations from the Pine Ridge in western Nebraska, though no historical dates were noted. Finally, one population in Johnson (Austin & Emmel 1998; Warren 2005; Davenport 2018; Butterflies of America 2022) is noted from Port Roche, Saskatchewan, Canada.

Relativized comparisons of recent and historical *E. ausonides* records within *E. a. palaeoreios'* distribution are similar within portions of South Dakota but there are too few observations, and sometimes none, in western Nebraska as well as most of this subspecies' limited distribution in western North Dakota (Fig. 8). While many urban areas of the western United States have seen an increase in sampling effort of butterflies, several regions in *E. a. palaeoreios*' distribution have relatively few records in the last ten years, including in South Dakota and southwestern North Dakota (Appendix B). The lack of recent butterfly observations generally and recent *E. ausonides* occurrence data in particular is concerning for an endemic subspecies that shows declines in other portions of its range.

Euchloe ausonides transmontana

This is the most widespread subspecies of *Euchloe ausonides*, with a southwestern edge in the Sierra Nevada Mountains in Tulare County on the Kern Plateau in southern California, extending east and north across the Sierra Nevada Mountains and northern California, Oregon, eastern Washington, Nevada, western Utah, and western Idaho (Austin & Emmel 1998; Warren 2005; Davenport 2018; Butterflies of America 2022). While Guppy and Shepard (2001) consider Washington and central Oregon populations to be *E. a. mayi*, most authors consider these populations to be *E. a. transmontana*, and that latter arrangement is followed here (Warren 2005; Butterflies of America 2022; Pelham 2022b, 2022a).

Known extirpations of this subspecies from the Shapiro long-term monitoring dataset in California include populations at Washington and Lang Crossing in Nevada County (Forister et al. 2021; Fig. 5). Populations monitored at NABA sites in Oregon, Washington, and western Idaho all are in consistent decline, with survey counts in Oregon reduced by almost 100% over the last 30 years, and Washington and Idaho counts reduced by approximately 50% (Forister et al. 2023; Fig. 5). Even as early as 1959, some sites in central Washington noted the absence of *E. ausonides* due to the effects of cattle grazing (Hopfinger 1960, 1961). These declines in count data support the effort-standardized comparisons of GBIF records that show relatively fewer recent *E. ausonides* records across almost the entirety of this subspecies' distribution including almost all of the sagebrush-steppe landscapes of Nevada, Oregon, Washington, Idaho, and Utah (Fig. 8). In particularly well-sampled regions like northwestern

Washington, which has over 17,000 GBIF butterfly records, *E. ausonides* records are 75% less frequent since 2013 compared to previous years; in eastern Washington, with over 16,000 records, *E. ausonides* records are over 80% less frequent since 2013 (Appendix B). Some sections of middle British Columbia, despite having higher numbers of recent butterfly records, have no *E. ausonides* records in the last 10 years (Fig. 8). Only regions near the subspecies' type locality in central Nevada and the California-Nevada-Oregon border have more observations in the recent time period relative to the historical time period.

Current and Potential Threats - Summary of Factors for Consideration

The ESA states that a species shall be determined to be endangered or threatened based on any one of five factors (16 U.S.C. § 1533 (a)(1)): 1) the present or threatened destruction, modification, or curtailment of its habitat or range; 2) overutilization for commercial, recreational, scientific, or educational purposes; 3) disease or predation; 4) the inadequacy of existing regulatory mechanisms; and 5) other natural or manmade factors affecting its continued existence. In this case, *Euchloe ausonides* is threatened by four factors: present or threatened destruction, modification, or curtailment of its habitat; disease or predation; inadequacy of existing regulatory mechanisms; and other natural or manmade factors affecting its continued existence. Thus, all subspecies of the large marble butterfly warrant protection under the Act. The best available science shows that the large marble butterfly is threatened and likely to become endangered in its entire range in the foreseeable future.

Factor One: The Present or Threatened Destruction, Modification, or Curtailment of its Habitat or Range

The large geographic range of the large marble means that its populations are subject to a wide variety of threats that have reduced the amount of available habitat. This reduction includes a widespread loss of all the habitat elements required for *E. ausonides*' development, including larval and adult food sources as well as suitable pupation and diapause locations (Griesemer et al. 2021). The large marble butterfly has experienced significant habitat loss and fragmentation across its range in western North America in the last several decades due to urban and exurban development, conversion of grasslands to agriculture, degradation of habitat from overgrazing, targeted removal of Brassicaceae caterpillar food resources and varied plant species used as adult food resources, and changes to wildfire regimes, and these threats continue today. The primary natural habitats for *Euchloe ausonides* include open areas such as grasslands, freshwater marsh areas, riparian openings, forest openings, meadows, and fields (Scott 1986, 2020; Shapiro & Manolis 2007; Philip & Ferris 2016; Pyle & LaBar 2018). Many of these habitats have been fragmented or reduced, and entire ecosystem types within *E. ausonides'* range have been reduced in size by over 50%; these ecosystems, including the Intermountain Semi-Desert Grasslands, California Central Valley annual grasslands, California chaparral, and the Snake-Columbia shrub steppe are considered Vulnerable or Imperiled by NatureServe (Noss et al. 1995; Lichthardt & Moseley 1997; World Wildlife Fund 2012; NatureServe 2022d; USDA National Agricultural Statistics Service Cropland Data Layer 2022).

While *Euchloe ausonides* has been observed in heavily developed locations, the small habitat patches that occur in these fragmented landscapes may be too isolated from source populations of the butterfly for successful dispersal, especially if patches are more distant than typical adult female dispersal distances (Schtickzelle et al. 2006; Griesemer et al. 2021). The food plant specialization of *E. ausonides* further increases the isolation between suitable habitat patches compared to generalist species (Sverdrup-Thygeson et al. 2017). Smaller habitat patches may also be lower quality compared to larger patches if food sources are not adequate to support a population (Öckinger & Smith 2007). Conversely, habitat connectivity has been shown to increase the ability of rare species to move across landscapes and to maintain genetic diversity following reductions in population size (Jangjoo et al. 2016; Kormann et al. 2019).

Urbanization

Euchloe ausonides requires a diverse landscape with disturbed soil for mustard host plant germination as well as undisturbed habitat for pupation sites and nectar plants; urbanization has significantly decreased the availability of these open habitat types. Urban development directly removes habitat through activities including building and road construction as well as conversion of natural vegetation to turf grass. Between 1982 and 2017, over 18 million acres of forested land and over 13 million acres of pasture and rangelands were converted into developed land in the United States (U.S. Department of Agriculture 2020). Urbanization has been shown to decrease butterfly abundance and diversity in a number of environments, including areas of central and southern California within the range of *E. ausonides* (Di Mauro et al. 2007; Forister et al. 2010; Bonebrake & Cooper 2014), and has been implicated in the decline of other widespread imperiled butterfly species such as the monarch, *Danaus plexippus* (Brower et al. 2012).

Development has been particularly intense within the range of *Euchloe ausonides ausonides* in the San Francisco Bay and Sacramento Valley regions, with almost total urbanization surrounding the bay itself (Connor et al. 2002). This region includes some of the steepest declines and earliest extirpations of *E. ausonides ausonides* (Fig. 5). This development has continued even in recent years, replacing abandoned agriculture and remaining grasslands and shrublands with developed land (Soulard & Wilson 2015). Development has been found to be particularly influential in butterfly species richness declines in Art Shapiro's lower elevation Central California monitoring sites that fall within *E. ausonides ausonides'* range (Forister et al. 2010). In addition to Central California, many of the fastest growing cities in other parts of the western United States fall within the ranges of *E. ausonides* subspecies, such as *E. a. transmontana* and *E. ausonides coloradensis* in central Utah (Salt Lake City area), southern and central Idaho (Boise area), and the western Great Plains in Colorado (Denver and Boulder regions) (US Census Bureau 2020).

In addition to urban growth, development in "exurban" areas outside of urban or suburban areas—sometimes called rural residential developments—has increased at several times the rate of suburban or urban expansion (Theobald 2005). This development often occurs near semi-natural areas, protected lands, or regional natural resources, and is typically lower density than suburban or exurban development. These lower density developments take up more space than typical urban development and can destroy high quality habitat required by butterfly species like *Euchloe ausonides*. Other potentially threatening factors associated with exurban areas include off-road vehicle recreation, human-caused wildfires, and infiltration by invasive plant species. These factors can contribute to the reduction in habitat needed by *E. ausonides* through the destruction of larval and adult food plants and the fragmentation of habitat (Huntsinger 2009; Smith et al. 2009; Robinne et al. 2016; Balch et al. 2017).

Conversion of natural or otherwise open habitats to urban and exurban development continues to threaten *Euchloe ausonides* habitat. Many counties in the San Francisco Bay and Sacramento Valley regions of *E. a. ausonides'* range in California are currently in a housing shortage; it is estimated that the San Francisco Bay area will need almost 450,000 new houses by 2031 while the central Sacramento Valley area will need over 150,000 new houses by 2135 (Sacramento Area Council of Governments 2020; Association of Bay Area Goverments 2022). Meeting these housing demands will most certainly lead to further development in these regions. Populations in western states across the ranges of *E. a. transmontana* and *E. a. coloradensis* are also expected to increase between 2020 and 2040, including a 15% increase in Montana, 21% in Oregon, 25% in Idaho, 27% in Washington, 30% in Nevada, 32% in Colorado, and 35% in Utah (Cooper Center Demographic Research Group 2022). Housing development needed to meet these increases will undoubtedly destroy some currently open grassland habitats used by *E. ausonides*, as these habitat types are valued for development due to their open nature.

Conversion to Agriculture

Grassland, marsh, and riparian habitats are also often highly valued for agriculture, and conversion of these habitats to agriculture has reduced and fragmented the habitat available for *Euchloe ausonides* populations. Some regions have undergone extensive conversion from open grasslands to agriculture, including the Sacramento Valley of California in the core range of *E. ausonides ausonides*, which had over 70% of its natural habitats converted to various crops by the 1970s (Sleeter 2008; Soulard & Wilson 2015). In the range of *Euchloe ausonides transmontana*, the Columbia River Basin in Washington lost over 60% of its original shrub-steppe habitat by 1986 compared to estimates of land cover previous to European settlement (Dobler et al. 1996), and the Palouse grasslands of Washington and Idaho are almost entirely converted to agriculture or are otherwise altered, with only remnant patches remaining, mostly in steep canyons (Lichthardt & Moseley 1997). In *E. a. coloradensis'* range, extensive conversion to agriculture has occurred in the northern short grasslands of northern and central Montana (Stephens et al. 2008; Rashford et al. 2011).

While the rate of conversion of land to agriculture has slowed in recent decades in some areas, expansion continues in many parts of *Euchloe ausonides'* range and continues to threaten the open habitats and food resources this species requires. Regions with recent agricultural expansion include large sections of *E. a. transmontana* and *E. a. coloradensis'* range in central and northern Utah, central Montana, western Colorado, central Washington, northern Oregon, and parts of northern and central California (Lark et al. 2015). In addition to agricultural expansion itself, some modern agricultural practices threaten the continued survival of *E. ausonides.* Larger scale agricultural operations in particular negatively impact the abundance of host plant species, as there are fewer farm margins and hedgerows that provide forage and shelter for the species. Larger, more homogeneous crop areas have also been shown to use insecticides and fungicides more frequently and at higher rates compared to more areas with higher crop diversity (Nicholson & Williams 2021). The risks of exposure to various pesticides is described below (Factor Five: Other Natural or Manmade Factors Affecting Its Continued Existence).

Livestock Grazing

Grazing is common across private rangelands as well as Bureau of Land Management (BLM) and U.S. Forest Service (USFS) managed lands across the western United States. Grazing on rangelands, primarily by cattle, is one of the most widespread land uses in the western United States (Fleischner 1994). Public rangelands in parts of the West have been degraded for at least the last half century, and the cause of this degradation is largely attributed to overgrazing by ungulates, especially cattle (U.S. GAO 1988; Noss et al. 1995; Bureau of Land Management 2022*a*). The most recent assessment in 2020 found 54 million acres of BLM land fail to meet the agency's own land-health standards, and of the allotments that are failing, 72% of them have their failure attributed to livestock grazing (Bureau of Land Management 2022*a*); many of these areas are regions of high habitat suitability for *Euchloe ausonides* including southern Idaho and southwest Wyoming (Fig. 7). These land-health standards include minimum benchmarks to ensure sustainable landscape function, and are based on factors including water quality, soil health, species diversity, and habitat quality. In addition, cattle are not the only ungulates that degrade western lands—domesticated sheep and feral horses and burros are common in certain regions of the western United States. When including these animals in assessments, rangelands from *E. a. transmontana* and *E. a. coloradensis'* range across the West often had realized grazing rates above Appropriate Management Levels set by the BLM and the effects of overgrazing were especially heavy in riparian areas, one of the preferred habitats for *E. ausonides* (Kaweck et al. 2018).

Overgrazing and its associated habitat degradation have already affected large sections of *Euchloe ausonides* habitat in the western United States and continue to threaten the species today. *Euchloe* *ausonides* localities across the species' range in the Great Basin Shrub Steppe, Western Short Grasslands, Colorado Plateau Shrublands, and Snake-Columbia Shrub-Steppe occur on BLM-managed rangelands as shown in Figure 10 (World Wildlife Fund 2012; Bureau of Land Management 2022b, 2022c; Bureau of Land Management & State of Utah School and Institutional Trust Lands 2022; Bureau of Land Management, Oregon State Office 2022). In almost every state where BLM lands are managed and were evaluated, some of these *E. ausonides* observations occur on lands where the land heath evaluation criteria were not met during the 2020 assessment, and many others occur on lands that have not yet been evaluated.

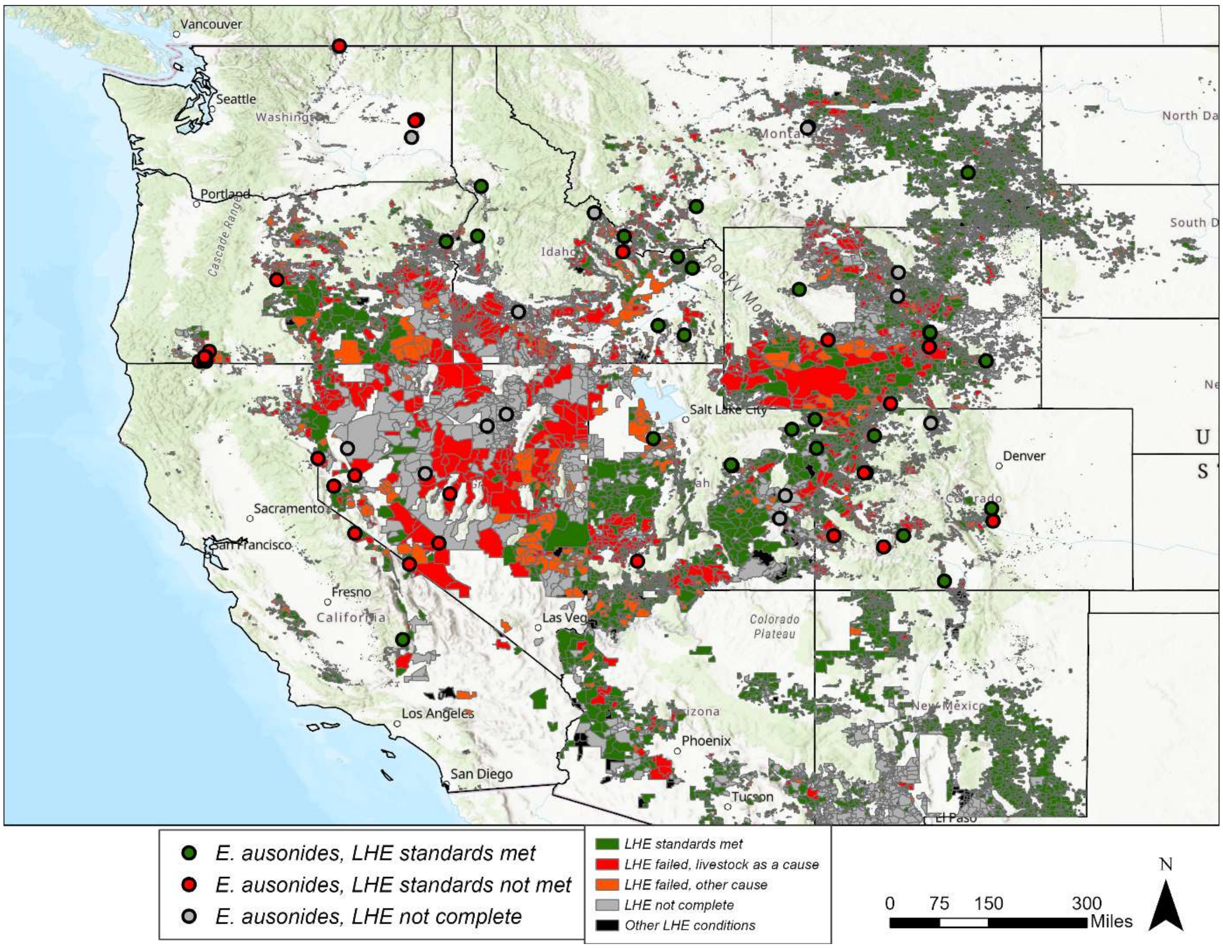

Figure 10. *Euchloe ausonides* observations on rangeland, color coded as pass/fail/not evaluated based on the 2020 BLM rangeland land health evaluation (LHE). Failing rangeland parcels are color coded by cause, with red parcels including livestock as a cause, with red parcels including livestock as a cause, and orange parcels listing only other causes. Data from BLM assessments (1997 – 2019), via FOIA request from Public Employees for Environmental Responsibility (PEER), available at BLM 2022*a*.

Excessively heavy livestock grazing seems to be at least partially responsible for the extirpation of *Euchloe ausonides insulanus* in British Columbia and for population declines of this subspecies in American Camp in Washington (Griesemer et al. 2021). In addition, as early as 1959 lepidopterists noted the absence of *E. a. transmontana* in central Washington locations due to the effects of overgrazing (Hopfinger 1960, 1961). Effects of overgrazing and related habitat degradation on butterfly populations include the destruction of native larval and adult food resources from livestock foraging or trampling, mortality of larvae and adults, and destruction of pupal diapause sites (Black et al. 2011). Plants in most areas of the Intermountain West, including the Great Basin Desert, have not evolved with grazing levels that exist on the landscapes today, and this has led to significant landscape degradation in some places (Noss et al. 1995; Morris & Rowe 2014). Erosion of fragile soils from livestock grazing, especially by cattle, is also a large concern as studies have found increased rates of soil erosion and sedimentation in streams concurrent with livestock grazing in areas across the Intermountain West (Jones 2000). This erosion removes topsoil in meadow and riparian areas that are preferred by *E. ausonides* and its food sources, resulting in a loss of available habitat for caterpillars and adults. Overestimation of stocking rates has led to some of these types of overgrazing effects; for example, this has been documented in Utah's High Uinta Wilderness, where *E. ausonides coloradensis* occurs (Carter et al. 2020; GBIF 2022*a*). Livestock can also facilitate the dispersal and germination of introduced plant species, especially cheatgrass, *Bromus tectorum*; this annual grass can compete with existing native plants that serve as caterpillar and adult food sources for *E. ausonides*, effectively reducing the quality and quantity of existing habitat (Mack 1981; Reisner et al. 2013).

Caterpillar Food Plant and Adult Nectar Plant Removal and Herbicide Use

Euchloe ausonides is a moderate host specialist, with its caterpillars able to feed on plants only within the mustard family, though its caterpillars can eat from plants of many genera within this family (see Table 1). These plant species include species that are both native and introduced to the United States, including 12 introduced species across the butterfly's range and 9 species in California alone (Graves & Shapiro 2003). Many of these larval host plants, both native and introduced, are targeted for removal across *E. ausonides*' range because they are considered noxious or unwanted plants by farmers and land managers. These plants are found across a variety of habitats but typically require shallow soil disturbance to germinate and so often favor disturbed sites including agricultural fields and margins, road edges, and open waste areas. Many of these species grow in areas where humans typically remove vegetation and are generally considered undesirable in most settings, including in rangelands or in landscape restoration projects that might otherwise prioritize host plants for imperiled pollinators. Several of these plant species compete with agricultural crops, or have other undesirable traits such as the toxicity of western tansymustard, *Descurainia pinnata*, to cattle (2012). A full list of *E. ausonides* caterpillar food plants that are considered rangeland and agricultural weed pests based on Whitson et al. (2012) is provided in Table 3. Mechanical removal and herbicide application to caterpillar food resources have been specifically noted as threats for *E. ausonides insulanus* (Foltz-Jordan et al. 2012; Griesemer et al. 2021).

Table 3. *Euchloe ausonides* caterpillar host plants considered undesirable and their human-associated germination sites, based on species descriptions in Whitson (2012:pp. 211, 213, 215, 225, 227, 233, 235).

Species	Targeted locations
Brassica nigra	roadsides, cultivated fields, other disturbed sites
Brassica rapa	cultivated fields, roadsides, waste areas
Descurainia pinnata	disturbed sites
Isatis tinctoria	roadsides, rangeland, cropland, other disturbed sites
Raphanus sativus	cultivated crops (especially cereal crops), waste areas
Sinapis arvensis	roadsides, cultivated fields, ditches, waste areas
Sisymbrium altissimum	small grain fields, rangeland, waste areas, roadsides

In addition to the nuisance nature of many *Euchloe ausonides* caterpillar food sources, three host plants are considered noxious by at least one state, and Dyer's woad (*Isatis tinctoria*) is included in many western states' highest level of pest classification (Table 4). In addition, many of *Euchloe ausonides'* documented nectar plants, especially *Cirsium* species (Table 2), are considered noxious in many states. These species and the relevant states with weed regulations include the majority of *E. ausonides*' distribution, and most large marble subspecies have multiple food plants targeted for removal.

Table 4. *Euchloe ausonides* caterpillar host plants considered noxious by state.

Brassica nigra	AZ (Class B)
Isatis tinctoria	AZ (Class A), CA (CCR 4500), CO (List A), NV (Class A), NM (Class A), ID, MT (Class 1A), OR (Class B), WA (Class A), WY
Sinapis arvensis	AK (Class B), AZ (Class A)

Continued removal of these species either by mechanical removal or herbicide application not only directly destroys existing habitat by removing potential caterpillar and adult food sources but can also cause caterpillar mortality if plants are killed while caterpillars are in the middle of developing, as was the case for *Euchloe ausonides insulanus* on Lopez Island in 2005 (Griesemer et al. 2021). Furthermore, if these plants are the only nectar source in degraded habitats or agricultural edges, removing these plants will remove crucial caterpillar and adult food sources, as has been shown in butterfly and other pollinator populations adjacent to agriculture (Moreby & Southway 1999; Nicholls & Altieri 2012). Finally, even if host plants are not subjected to direct herbicide application, they may still be subject to non-target herbicide exposure when herbicides for nonnative mustards are applied within agricultural areas during conditions where pesticides drift to non-target locations.

Use of herbicides for weed suppression in and near crop fields and in other land uses has likely reduced the availability of host plants for many Lepidoptera, including *Euchloe ausonides,* and continues to degrade the quality of remaining habitat via impacts to vegetative growth and flowering. In agriculture, genetically-modified herbicide-resistant crops have expanded the use of herbicides to control weeds in crop fields, from about 35 million pounds annually in 1994, to 276 million pounds in 2014 (Benbrook 2016). It is likely this number is even higher today (Clapp 2021). This herbicide use has the potential to remove large amounts of potential lepidopteran food sources from around crop edges. For example, more frequent use of glyphosate—a non-selective herbicide that kills many plants—resulted in a 58% decline of milkweed stems in the Midwest (Pleasants & Oberhauser 2013) and this loss of caterpillar food plants has been identified as a major driver in the decline of monarch butterflies. Because glyphosate also kills mustards, an increase in its use similarly threatens *Euchloe ausonides* populations across the butterfly's range, especially for *E. a. ausonides*, *E. a. transmontana*, *E. a. coloradensis*, and *E. a. palaeoreios*, which occur in areas of heavy glyphosate use (Fig. 11).

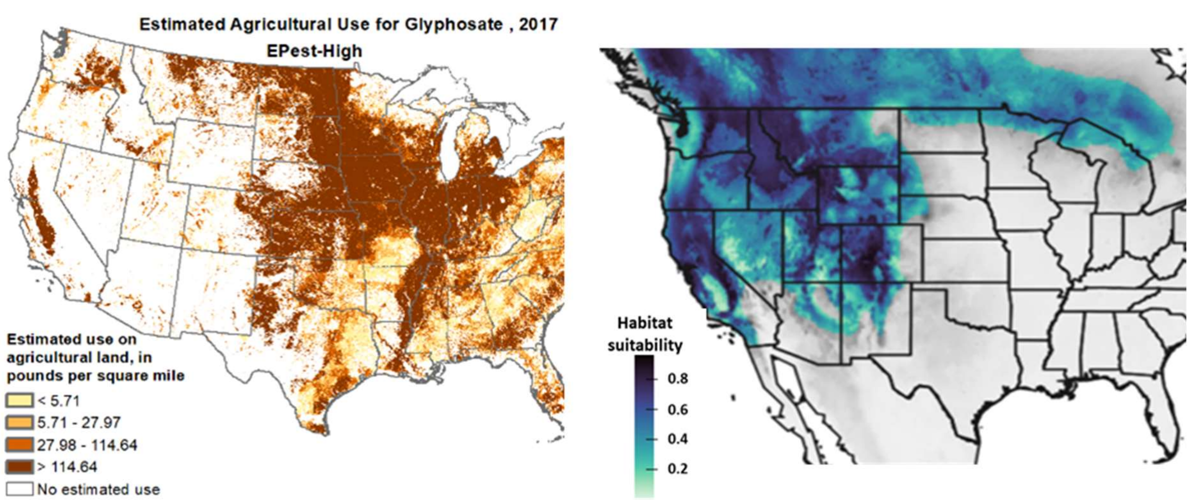

Figure 11. Glyphosate use in 2017 (the most recent year complete data are available; left), and continental U.S. habitat suitability for the range of *Euchloe ausonides* (right). Areas of particular concern within the large marble's range include Central California, eastern Washington and northeast Oregon, Montana, and the western portions of North Dakota, South Dakota, and Nebraska. Data from the USGS Pesticide National Synthesis Project, available at USGS (2022).

Use of the plant growth regulator herbicides dicamba and 2,4-D is also on the rise since the development of herbicide-resistant crop varieties in corn, soy, and cotton—crops that are grown within the ranges of *E. ausonides ausonides* and *E. ausonides transmontana*. Bohnenblust et al (2013) performed a toxicity experiment with painted lady butterflies (*Vanessa cardui*) feeding on thistle host plants, and found that plants sprayed with dicamba had stunted growth that in turn limited feeding for *V. cardui* larvae in the study. They also found that thistle plants sprayed with dicamba had reduced nitrogen content, showing that host plants treated with herbicides can reduce quantity and also quality of food resources for larvae. Dicamba and 2,4-D herbicides are highly volatile and prone to drift, which threatens the availability and quality of food sources for *E. ausonides* in areas where they are applied; many non-target plants are sensitive to drift-level doses of these plant growth regulators at certain times in their life cycles (Carlsen et al. 2006*a*, 2006*b*; Mehdizadeh et al. 2021). For example, one review of drift studies found that glyphosate rates needed to be below 5 g active ingredient per hectare to avoid harming 95% of nontarget species, which is 0.6% or less of commonly used application rates (U.S. Environmental Protection Agency 2016; Cederlund 2017).

Even in non-agricultural settings, the larval host plants, and sometimes the nectar plants of *Euchloe ausonides*, are targeted with herbicide applications as well. Similar to disturbed agricultural edges, Brassicaceae plant species required by *E. ausonides* are often found along roadsides and rights of way, irrigation ditches, golf courses, and residential lawns. The broadcast application of a non-selective herbicide can indiscriminately reduce floral resources and host plants (Smallidge & Leopold 1997), in some cases significantly altering the plant community composition in open spaces.

Changes in Historical Wildfire Regimes

The large geographic range of *Euchloe ausonides* includes habitats with a range of historical fire regimes. In most grasslands, prairies, and mountain ranges, natural or anthropogenic fire sources historically maintained open, light-filled patches of habitat and prevented woody encroachment, and these disturbances can be beneficial to butterfly communities in these areas (Waltz & Wallace Covington 2004). These fires create the open habitat preferred by *E. ausonides* adults and often facilitate the germination of its required Brassicaceae host plants and can also increase the diversity of adult nectar sources (Keeley & Keeley 1987; Rudolph & Ely 2000; Huntzinger 2003). The more recent suppression of fire and loss of open habitat or required conditions for caterpillar food sources has been cited in the decline of multiple endangered species from within the range of *E. ausonides* and beyond, including *Euphydryas editha taylori* and *Icaricia icarioides fenderi*, *I. shasta charlestonensis*, and *Plebejus samuelis* (Clough 1992; Clark & Wilson 1996; The Xerces Society et al. 2002; King 2003; Andrew et al. 2013), as well as the regal fritillary (*Argynnis idalia*), which is currently under review by the U.S. Fish and Wildlife Service (U.S. Fish and Wildlife Service 2015). Large wildfires have declined steadily across the grasslands of Mediterranean California since at least the mid-1980s, an additional threat to the majority of *E. ausonides ausonides*' range and habitat (Dennison et al. 2014).

In addition to central California, changes in wildfire regimes in other regions threaten *Euchloe ausonides transmontana* and *E. a. coloradensis* habitat. In most forested regions of the western United States the frequency of large fires is increasing, including in *E. a. transmontana* and *E. a. coloradensis* habitats of the Sierra Nevada, Klamath, and Rocky Mountains (Fig. 12) (Westerling et al. 2006; Dennison et al. 2014). Effects of larger, high severity fires, sometimes referred to as "stand-replacing" fires, at and near the soil include soil dehydration, loss of organic material, and degradation of soil texture as well as the death of soil microorganisms, seeds, and animals in shallow soil (Miller et al. 2009; Fenstermaker 2012). Insect mortality includes diapausing insects in shallow soil (Cane & Neff 2011), and *E. ausonides* pupae would likely be killed by a high intensity fire, since this species pupates in shallow soil. These fires also tend to make the forest landscape more homogeneous, reducing opportunity for patchy forest openings and reducing plant and pollinator diversity (Ponisio et al. 2016; Cassell et al. 2019). Depending on the local plant community before fires, these large fires may not increase the availability of annual mustards used by *E. ausonides*, contrary to standard expectations. For example, one study of Cascade forests that had experienced recent fires found that annual herbs still accounted for less than 1% of herbaceous plant cover two years following a second fire; instead, perennials were primarily responsible for changes in early-seral plant dominance (Halpern & Antos 2022). These changes to fire frequencies and intensities can result in a loss of caterpillar and adult food sources as well as pupal diapause sites, endangering the sustainability of *E. ausonides* populations across large sections of the western landscape.

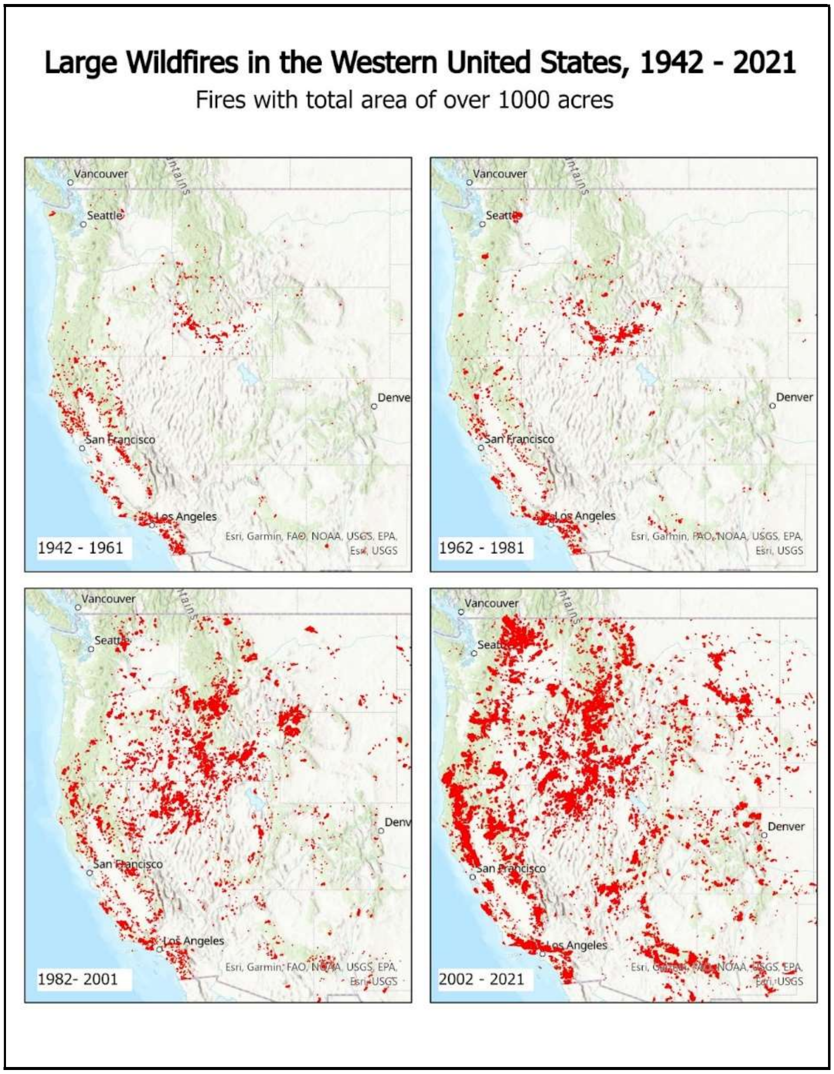

Figure 12. Perimeters of large fires (> 1000 acres) in four 20-year periods. Data from National Interagency Fire Center (NIFC 2021).

On the eastern edge of the species' range, *Euchloe ausonides palaeoreios* populations are also threatened by changes in fire regimes. This subspecies is associated with climax conifer forests on the western edge of the Great Plains (Johnson 1976). These forests are relicts of the last ice age, disconnected from the Rocky Mountains to the west and distinct in ecology from the plains to the north (Johnson 1975). Previous to European settlement, frequent low severity fires with both natural and manmade ignition sources were responsible for maintaining a semi-open, park-like structure of ponderosa pine stands with surrounding grasslands (Murphy 2017). As with other forested regions, settlement by Europeans was associated with fire suppression, leading to woody encroachment in grasslands (Brown & Sieg 1999; Murphy 2017). Woody encroachment and loss of grassland habitat in this region is likely to reduce the available habitat for *E. a. palaeoreios* larvae and adults. More recently, increasing temperatures, prolonged drought, and earlier spring snowmelt have influenced fire regimes across the West; in the Black Hills of South Dakota and Wyoming, this includes larger fires and prolonged fire seasons that overlap with *E. a. palaeoreios'* development and reproduction (USDA Forest Service 2013; Murphy 2017). Similar to other forested regions, high intensity fires are likely to threaten overwintering individuals or flying adults with mortality and may also lead to the long-term degradation of the soil and plant communities that this endemic subspecies requires.

The likelihood of longer fire seasons and larger and hotter fires is expected to increase in the coming decades and is likely to endanger *E. ausonides* populations across the species' range in the foreseeable future. Negative consequences of these changes to fire regimes that are already occurring, including loss of larval and adult nectar plants and destruction of larval diapause sites, are likely to grow as the frequency and intensity of fires increases in the coming decades (Fincher 2012). Across forested areas of the northern United States and Canada, including areas within the range of *Euchloe ausonides transmontana*, *E. a. coloradensis*, *E. a. ogilvia*, *E. a. mayi*, and *E. a. palaeoreios*, larger, more intense, and more destructive fires are expected to continue, posing an ongoing threat to *E. ausonides* populations. Liu et al. (2013) found indexes of drought and of fire potential were expected to increase across *E. a. palaeoreios'* range in the Great Plains in every season because of increased temperatures and decreased relative humidity. Fire potential for the rest of the western United States in *E. a. transmontana* and *E. a. coloradensis'* ranges also increased in the summer and autumn, and other studies have predicted larger fires in mixed conifer forests within *E. a. transmontana*'s range (Liu et al. 2013; Cassell et al. 2019). In Alaska, French et al. (2015) used a separate index and found that future fire danger is expected to increase across the Alaskan tundra within the range of *E. a. ogilvia* based on a range of climate models. Other models focusing on southern Canada also predict increased likelihood of fires and wildfire size in *E. a. mayi*'s range (Nitschke & Innes 2008; Wang et al. 2020).

In contrast to populations in forested regions, *Euchloe ausonides transmontana* and *E. a. coloradensis* populations that are found in arid, low elevation sites across the Intermountain West are also threatened by changes to historical fire regimes. In most shrub steppe habitats, including the Great Basin, Snake and Columbia River Basins, and Colorado Plateau, historical fire frequencies ranged between 30 and 100 year return intervals (Menakis et al. 2003). The combination of overgrazing reducing the availability of perennial forbs, coupled with the introduction of nonnative annual grasses and forbs, especially *Bromus tectorum*, has led to an increase in fine fuels in these areas that are easily ignitable by natural or man-made sources (Brooks & Chambers 2011; Morris & Rowe 2014). These fine fuels create a grass/fire cycle with larger, recurring fires as often as every few years in parts of *E. a. coloradensis'* range in Wyoming and *E. a. transmontana*'s range in Idaho (Fig. 9) (Whisenant 1990; Brooks & Pyke 2001). While annual plants like the Brassicaceae plants required by *E. ausonides* caterpillars may be part of this annual plant community, all life stages of *E. ausonides* are susceptible to mortality by fire. Frequent fires may be sufficient to disrupt local seed banks or plant life and butterfly annual life cycles, and may also reduce the availability and diversity of flowering forbs and shrubs used as adult food sources (Brooks et al. 2004; Underwood et al. 2019). Limited studies have been conducted in this region, but Love and Cane (2016) found a reduction in bee species richness, including a loss of several specialist species, immediately after a large wildfire within *E. a. transmontana's* range in southwest Idaho. This loss in species diversity is likely due to mortality of nesting females in shallow (<10 cm) soil and roosting males that are often above ground. Developing and diapausing *E. ausonides* individuals that are immobile or sedentary and are close to ground level would similarly be vulnerable to mortality by heat and desiccation caused by wildfires in these areas. However, studies monitoring pollinator communities in the years following fires in these regions suggest that species richness of pollinators may increase if flowering forb species are present and fire does not return too quickly (Kral et al. 2017; Smith DiCarlo et al. 2019).

Factor Three: Disease or Predation

Pierid butterflies are predated by a number of native and nonnative animals as part of western food webs. In addition to native predators including numerous arthropods and vertebrates, *E. ausonides* is vulnerable to nonnative predators or parasitoids with which it has not evolved, as has been shown in other lepidoptera species (Van Driesche et al. 2003; King 2008). The nonnative Pieridae butterfly species *Pieris rapae* is a major pest of Brassicaceae crops in many places, including the United States, and multiple biocontrol agents have been released to manage its populations (Parker & Pinnell 1972; Van Driesche 2008; Harvey et al. 2010; Bryant et al. 2014); those that have established pose a threat to the continued existence of *E. ausonides* where they occur. Some of the most common biocontrol agents of *P. rapae* found in agricultural settings today include the parasitic wasps *Cotesia rubecula* and *C. glomerata* (Hymenoptera: Braconidae), the latter of which also feeds on the European Pieridae butterfly *P. brassicae* (Le Masurier & Waage 1993). While these two *Pieris* species are the primary biocontrol targets of these parasitoids, *C. glomerata* likely also parasitizes other butterfly species in the family Pieridae (such as *E. ausonides*) and has been hypothesized to be partially responsible for local extirpations of the native Pieridae butterfly *P. oleracea* (= *napi*) *oleracea* (Harris 1829) in New England based on laboratory choice and no-choice feeding experiments, field studies, and simulation models (Benson et al. 2003; Van Driesche et al. 2003; Keeler et al. 2006).

The ability of *Cotesia* parasitoid species to parasitize *Euchloe ausonides* is currently unknown. However, in one study on the related parasitoid *C. vestalis*, known primarily for parasitizing *Plutella xylostella* moths, females stung 27 lepidoptera species, including three species stung at the same rate as its primary biocontrol host *P. xylostella*, and were able to successfully form cocoons in 15 species (Hiroyoshi et al. 2017). These parasitoids have also been shown to be very common in nonnative mustard populations on the edges of crop areas and roadsides (Van Driesche 1988; Van Driesche & Bellows 1988), and specimens *of C. glomerata* from within the range of *E. a. ausonides* come from as early as 1983 (SCAN 2023). The related parasitoid *C. plutellae*, also used to control *P. xylostella*, has also been shown to lay eggs on *Pieris rapae* and other non-target Lepidoptera (Cameron & Walker 1997). *Cotesia plutellae* is also on the Animal and Plant Health Inspection Service (APHIS) list of biocontrol agents exempted from the standard permitting process, so we are unable to assess how widespread these non-native parasitoids are in *E. ausonides'* range (Animal and Plant Health Inspection Service 2020a). The relatively large taxonomic breadth of caterpillar hosts and their abundance across the landscape make *Cotesia* wasps a potentially devastating threat to *E. ausonides* populations, especially in *E. a. ausonides'* range in California's Sacramento Valley and Central Coast, both of which have substantial acreage of Brassicaceae crops and abundant *P. rapae* populations (Le Strange et al. 2010; Chambers et al. 2015; Garvey 2022).

The phenology of *Euchloe ausonides* may also increase its risk of attack by *Cotesia* parasitoids in some locations. Benson et al. (2003) hypothesize that variable diapause rates in *Pieris oleracea* populations may have exposed southern populations that have larger second broods to greater parasitism than populations where a larger percentage of first generation pupae diapause until the next season. This life history feature is also relevant to *E. ausonides*, as *E. a. ausonides* populations in California were historically bivoltine but the second generation of these populations has largely vanished (Shapiro & Manolis 2007). While the exact phenology of *C. glomerata* in the western United States has not been documented, adults have been observed as late as October 1, and it is likely active much of the year in low elevation regions, much like its typical *P. rapae* hosts (GBIF 2022*c*). If *C. glomerata* parasitoids are abundant during *E. ausonides* second broods, especially in central California, these wasp species pose a significant threat to *E. a. ausonides* populations.

In addition to *Cotesia* wasps, species of wasps in the genus *Trichogramma* (Chalcidoidea: Trichogrammatidae) also parasitize the eggs of hundreds of Lepidoptera species and other insect groups to a lesser extent (Zucchi et al. 2010). Among these species, *T. brassicae* is distributed in the United States as a biocontrol agent for *P. rapae* by multiple companies (Rincon-Vitova Insectaries 2021; Buglogical Control Systems 2022). *Trichogramma brassicae* has a broad documented host range including at least three *Pieris* species and Lepidoptera in 13 families (Babendreier et al. 2003; Polaszek 2010). While the ability of *T. brassicae* to parasitize *Euchloe ausonides* has not been tested, the broad documented host range and high fitness of offspring from many hosts suggests *E. ausonides* is threatened by broadscale releases of *Trichogramma* that are recommended for biological pest control (Knutson 1998). Finally, similar to *Cotesia pluetellae*, five *Trichogramma* species, including *T. brassicae* and *T. evanescens* (also used to control *Pieris rapae*) are included on the APHIS list of biocontrol agents exempted from the standard permitting process, so their use is essentially unrestricted and we are unable to assess how widespread these non-native parasitoids are used within *E. ausonides'* range (Animal and Plant Health Inspection Service 2020*a*).

Factor Four: The Inadequacy of Existing Regulatory Mechanisms

Regulatory Mechanisms Protecting the Species' Habitat

While the island marble butterfly, *Euchloe ausonides insulanus*, and its Critical Habitat are protected by the Endangered Species Act, this particular subspecies currently persists in a single known population and has been observed on a total of three adjacent islands in the Southern Gulf Islands archipelago (Griesemer et al. 2021). Because all but a single *E. ausonides* population remain unprotected by the Endangered Species Act, the habitat essential to the continued survival of the species is not protected from destruction or adverse modification throughout its range in the United States. The inclusion of *E. a. palaeoreios* in the Nebraska State Wildlife Action Plan's Tier 2 Species of Greatest Conservation need offers no formal protections of any kind, and no subspecies other than *E. a. insulanus* is protected by the Endangered Species Act or any state endangered species legislation.

Regulatory Mechanisms Protecting the Species from Introduced Biocontrol Agents

Existing regulations regarding the movement of biocontrol agents, controlled by the Animal and Plant Health Inspection Service (APHIS), are inadequate to protect *Euchloe ausonides* from non-native predators that are used to control pest caterpillars. Proposed in 2017 and enacted beginning in August 2019, APHIS revised the regulations that govern the movement of plant pests to "align regulations with current policies, remove obsolete requirements, streamline the permit process for low risk organisms, and update requirements for the import of foreign soil" (Animal and Plant Health Inspection Service 2017, 2019*a*). These revisions included a list of organisms that became exempt from permitting processes typically required to import or move these organisms across state lines. Currently this list includes three *Cotesia* and five *Trichogramma* wasp species that use Lepidopteran eggs or caterpillars as hosts, and in some cases are specifically released to control the pest butterfly *Pieris rapae*, a Pierid relative of *E. ausonides* (Animal and Plant Health Inspection Service 2020*a*). These biocontrol agents have the potential to reduce populations of both introduced pest Pierids and native species such as *E. ausonides*. The inability to track the transport of these non-native predators into agricultural areas within the range of *E. ausonides* leaves these butterfly populations unprotected, including the federally endangered island marble butterfly, *E. a. insulanus*, as we are currently unable to assess the scale of this threat due to the lack of information regarding use. This is also particularly concerning for the type subspecies *E. a. ausonides*, which is now absent from the largely agricultural northern Sacramento Valley in California and may be negatively impacted in other parts of its range where it still occurs.

Regulatory Mechanisms Protecting the Species from Pesticides

Existing regulations are inadequate to protect *Euchloe ausonides* from pesticides. The Environmental Protection Agency (EPA) screens pesticides for harm to pollinators before registration for use, but there are several flaws in the existing risk assessment process. The EPA uses the European honey bee (*Apis mellifera* L.) as the standard test organism to assess lethality of pesticides to pollinators and other terrestrial insects. However, the honeybee is often not an adequate surrogate for butterflies; native butterflies are from a different order of insects and diverge in their physiology, behavior, and life history in important ways that could affect responses to pesticides (Pisa et al. 2015; Braak et al. 2018). Standard Tier I screening with adult honey bees may miss key differences in Lepidopteran responses and sensitivity to pesticides. While one of the goals for using *A. mellifera* is to gather relevant information on the potential effects of a pesticide that can be provided for non-target Lepidoptera like *E. ausonides*, these distinct differences in biology severely limit the ability infer the danger of pesticides to imperiled butterflies (U.S. Environmental Protection Agency et al. 2014).

Several other gaps in regulation expose *Euchloe ausonides* to damaging effects of pesticide exposure. First, EPA does not require examination of sublethal effects as a standard part of the assessment process for pesticide risk. Sublethal effects are effects of a pesticide that do not kill the insect immediately, but can have long-term consequences for the exposed individual such as reduced lifespan, fecundity, and body mass as well as delayed development and altered behavior (Desneux et al. 2007). In addition, there is limited regulation and registrations of adjuvants and co-formulants in pesticides, in spite of evidence that these substances can produce lethal and sublethal effects in insects (Ciarlo et al. 2012; Mesnage & Antoniou 2017; Straw et al. 2021; Wernecke et al. 2022). Finally, EPA's risk assessment does not examine the potential for synergistic effects of multiple pesticides and instead only requires testing one active ingredient at a time, when in reality, we know that butterflies are exposed to mixtures of pesticide products as the norm in agricultural sites, in cities, and even in natural areas. Halsch et al. (2020) found an average of nine and up to 25 different pesticides on milkweed leaves collected from agricultural, metropolitan, and refuge sites within the Central Valley of California. These important gaps in assessing risk during registration of a pesticide pose a threat to *E. ausonides*, as well as other butterflies.

Lastly, federal pest programs fail to appropriately assess risk to protected species. The U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) does not adequately consider harm to endangered species caused by insecticide spraying across millions of acres of western grasslands (Xerces Society & Center for Biological Diversity 2022). These widespread insecticide applications are approved across the range of *E. ausonides* and have the potential to dramatically impact the species.

State Wildlife Agency Management Authority to Conserve Insects

Euchloe ausonides occurs in sixteen western states, yet in seven of these states, the state wildlife agency lacks authority to conserve insects, typically because the state definitions of wildlife are limited to only other types of animals, presenting a major gap in the U.S. wildlife conservation regulatory structure. The states within *E. ausonides* range that currently lack insect or terrestrial insect management authority include: Alaska, Colorado, Nevada, New Mexico, Oregon, Utah, and Wyoming. As such, there is no mechanism for state wildlife agencies in these states to proactively conserve *E. ausonides* in order to avoid ESA listing.

Factor Five: Other Natural or Manmade Factors Affecting its Continued Existence

Pesticide Use

Pesticides are a factor contributing to the decline of pollinators (Goulson et al. 2015), and several studies have pointed to pesticides as a cause of butterfly declines in both the United States and Europe (Gilburn et al. 2015; Forister et al. 2016; Van Deynze et al. 2023). There are multiple pesticide exposure routes for *Euchloe ausonides*. They can be exposed as larvae, with pesticides applied onto them directly (contact exposure), with pesticides applied on their host plants that they crawl on (indirect contact exposure), and with pesticides applied on their host plants that they consume (oral ingestion). Pesticides sprayed in nearby agricultural fields can also drift onto adults, larvae and host plants. Systemic pesticides present in soils from seed coatings, or application of granules or soil drenches can be transported via runoff and subsurface flow to host plants that take up the pesticides and distribute them to roots, stems, leaves, flowers, pollen, nectar and honeydew. Adult butterflies can be exposed by contact to foliar applications or drift deposition, or by drinking contaminated nectar and honeydew (Braak et al. 2018). Each of these exposure pathways threatens *Euchloe ausonides*.

Pesticide exposure is an ongoing threat to *Euchloe ausonides* populations across its range. Pesticides, including insecticides, herbicides, and fungicides, are used throughout *E. ausonides'* range for a variety of pest control applications. The broad geographic distribution of *Euchloe ausonides* exposes individuals to a wide array of pesticides that vary according to the location. In urban locations, such as in *Euchloe ausonides ausonides*' range in central California, large amounts of pesticide are used for a variety of public health, cosmetic, nuisance, and economic pests. This is similarly true for *E. a. transmontana* populations near Salt Lake City, Utah, and surrounding urban areas; Boise, Idaho; and for *E. a. coloradensis* populations in the foothills of the Front Range in Colorado.

In other locations, populations of *Euchloe ausonides* are threatened by broad-scale exposure to agricultural pesticides. Historically, many populations of the type subspecies *Euchloe ausonides ausonides* in the Sacramento Valley occurred in regions with intense and varied agriculture. The application of neonicotinoids to crops in this region has already been implicated in the decline of butterfly community diversity at sites that historically included this subspecies and from which it is now extirpated (Forister et al. 2016). In the range of *E. a. transmontana*, the Palouse Grasslands have been almost entirely converted to agriculture, and the Snake-Columbia shrub-steppe has also had large portions of land devoted to agriculture including row crops, vegetables, and orchards (United State Geological Survey 2016). Pesticides are commonly applied in almost all of these agricultural land uses by a variety of methods, including foliar applications, through irrigation systems (i.e. chemigation), and to the soil (i.e. the planting of treated seed)., Much of *E. a. mayi*'s range in Alberta and Saskatchewan also includes large sections of row crop agriculture (Agriculture and Agri-Food Canada 2019).

Finally, in other regions in large sections of natural landscapes, pesticides are sprayed to control herbivorous pests that either compete with domesticated livestock or defoliate trees of economic value. In the United States, *Euchloe ausonides transmontana* and *E. a. coloradensis* can be found across western rangelands where pesticides are used to manage native species such as grasshoppers and Mormon crickets (*Anabrus simplex*) (see Fig.15 in Insect Growth Regulator section). Further north in Canada, some *E. a. mayi* populations are exposed to the biological pesticide *Bacillus thuringiensis* var. *kurstaki* (*Btk*) used to control *Lymantria dispar* infestations in both British Columbia and Ontario (Linton & Norris 2021; Arrais 2022).

For each pesticide, risk is determined by both hazard and exposure. Hazard can include direct and indirect harm and both lethal and sublethal endpoints, including impacts to reproduction, growth and development, and behavior. Exposure routes include contact and oral exposure that can happen in a variety of scenarios throughout a butterfly's lifecycle. Insecticides are designed to kill insects directly, and have the greatest potential for lethal and sublethal harm to *Euchloe ausonides*. Herbicides can both indirectly affect Lepidoptera by removing or degrading floral resources and host plants, and directly affect Lepidoptera by causing lethal or sublethal effects to butterflies (Stark et al. 2012; Bohnenblust et al. 2013; Schultz et al. 2016). Research on fungicides shows that certain active ingredients and formulations can have direct lethal and sublethal effects in Lepidoptera (El-Kholy et al. 2018). Pesticides also contain co-formulants which are not specifically tested for their lethality, many of which are also toxic to butterflies (Stark et al. 2012; Freydier & Lundgren 2016).

Pesticide use data

Pesticide use is not well tracked. Within the U.S., California is the only state that requires relatively comprehensive pesticide use reporting for agricultural uses. In total, there were 116 million pounds of pesticide product used for agriculture in *Euchloe ausonides ausonides'* range across 23 counties in California in 2018, with 671 different products used (CA DPR 2022). It should be noted that these data are an underestimate, as seed coatings in agriculture, as well as home, industrial, and institutional uses, are not included in data collected by California Department of Pesticide Regulation. We will refer to the

CA data throughout this section to reiterate the heavy usage within the range of *Euchloe ausonides ausonides*.

Toxicity of pesticides to native (non-pest) Lepidoptera species are not well studied. However, pesticide manufacturers sometimes conduct tests of their products on pest Lepidoptera, including the related Pierid butterfly *Pieris rapae*, so we can gain biologically relevant insights into the vulnerability of *Euchloe ausonides* to specific pesticides or pesticide families from research on other butterflies and moths. A number of these studies look at pesticide risk to Lepidoptera; some are lab studies while other studies assess impact in field settings where *Euchloe ausonides* individuals could be found.

Neonicotinoids

Despite being identified by the Environmental Protection Agency as likely harming three quarters of endangered species (EPA 2021a, 2021b, 2021c), and being banned and restricted in Europe and Canada, respectively, there are limited U.S. regulations on neonicotinoids. Neonicotinoids are the most widely used group of insecticides in the world, comprising roughly 25% of the agrochemical market (Jeschke et al. 2011). The majority of neonicotinoid use is as seed coatings, especially for crops like cotton, wheat, corn, and soy but also including crops such as squash, tomatoes, broccoli, and sunflowers. Crops only take up 2-20% of the pesticide in the seed coatings (Sanchez-Bayo & Goka 2014), and the rest is available for transport into the surrounding environment. Seed coatings release the neonicotinoids into the soil where they can be transported via runoff and subsurface flow. When transported off crop fields, they can end up in field margins where butterfly host plants commonly grow, and the neonicotinoids can be taken up by the host plants, creating exposure routes to feeding larvae and nectaring butterflies.

Neonicotinoids have significant lethal and sublethal impacts on butterflies. Forister et al. (2016) found a negative association between butterfly population sizes and increasing neonicotinoid applications at Shapiro butterfly monitoring sites that all historically included *Euchloe ausonides ausonides*. In addition, Gilburn et al. (2015) observed population sizes of 17 widespread butterfly species in the UK over 27 years, and found a strong negative correlation between population sizes and neonicotinoid use. These studies suggest that neonicotinoids may be driving population declines of *E. ausonides* in areas of heavy neonicotinoid use.

Mechanistic studies also highlight the negative effects of neonicotinoids on lepidopteran larvae. A 2015 study examining the effects of a soil-applied neonicotinoid at plant nursery relevant application rates found significant mortality in both monarch and painted lady larvae (Krischik et al. 2015). Exposure to environmental levels of agricultural neonicotinoids has been shown to result in reduced survival of monarch caterpillars and has led to a decline in abundance of other insects found on agriculture field-adjacent milkweed (*Asclepias*) plants (Pecenka & Lundgren 2015; Knight et al. 2021), suggesting this is a possibility for other butterfly species (like *E. ausonides*) that use plants found in agricultural margins. Another study testing lethality of one neonicotinoid, imidacloprid, on monarch adults found that survival was significantly reduced when adults were fed nectar and pollen with imidacloprid levels consistent with agricultural and wildflower concentrations.

Neonicotinoids have been documented moving off crop field areas and into crop margins with butterfly food sources and can remain there long after application. Botías et al. (2016) found that neonicotinoid use in oilseed rape fields contaminated nearby wildflowers with even higher total neonicotinoid concentrations than the crop they were supposed to protect. The highest concentration in leaves they found was 106 ppb thiamethoxam in *Cirsium vulgare* (a relative of one of the adult food sources of *Euchloe ausonides*), compared to the average of 4.2 ppb in treated crops. The authors also found imidacloprid in leaf samples, even though it had not been applied at that field in three years. They also found thiacloprid and acetamiprid in leaf samples, even though those neonicotinoids had never been applied in that field. This study suggests that neonicotinoids can persist in the soil for long periods of time, with non-target plants in field margins continuously taking up concentrations, and neonicotinoids can travel through runoff, subsurface flow, and dust off from the planting of treated seeds from agricultural field to field, contaminating non-target plants on the way.

Negative effects of neonicotinoid exposure have been demonstrated in Pieridae relatives of *Euchloe ausonides*. Whitehorn et al. (2018) found that *Pieris brassicae* larvae feeding on Brassicaceae host plants watered with field realistic levels of imidacloprid had reduced pupal development time and reduced adult forewing length. Sublethal effects can have harmful repercussions on individuals and whole populations exposed to pesticides. Altered development times run the risk of population asynchrony that could decrease mating success (Jones & Aihara-Sasaki 2001), and reduced size in butterflies could result in reduced fitness, reduced fecundity in females, and reduced mating success in males (Boggs & Freeman 2005).

The abundance of *Euchloe ausonides* caterpillar food sources near agricultural areas combined with the prevalence of neonicotinoids in these locations places this butterfly at an increased risk of lethal or sublethal injuries from exposure, and this risk is likely to continue. Since their development, neonicotinoid use has increased in the US (Fig. 9). Within *E. a. ausonides'* range in California, 570 thousand pounds of neonicotinoid products were used in 2018 (CA DPR 2022) and this is an underestimate as this figure does not include any neonicotinoid coated seed that was planted in the state, nor does it include use of neonicotinoids for home or commercial landscaping. Other areas with high levels of neonicotinoid use include the Snake and Columbia shrub steppe in northern Oregon, Washington, and Idaho, all within the habitat of *E. a. transmontana*; portions of northern Montana in *E. a. coloradensis*' northern grassland habitat; and in the northern Colorado and western Nebraska region near *E. a. palaeoreios* (Fig. 13).

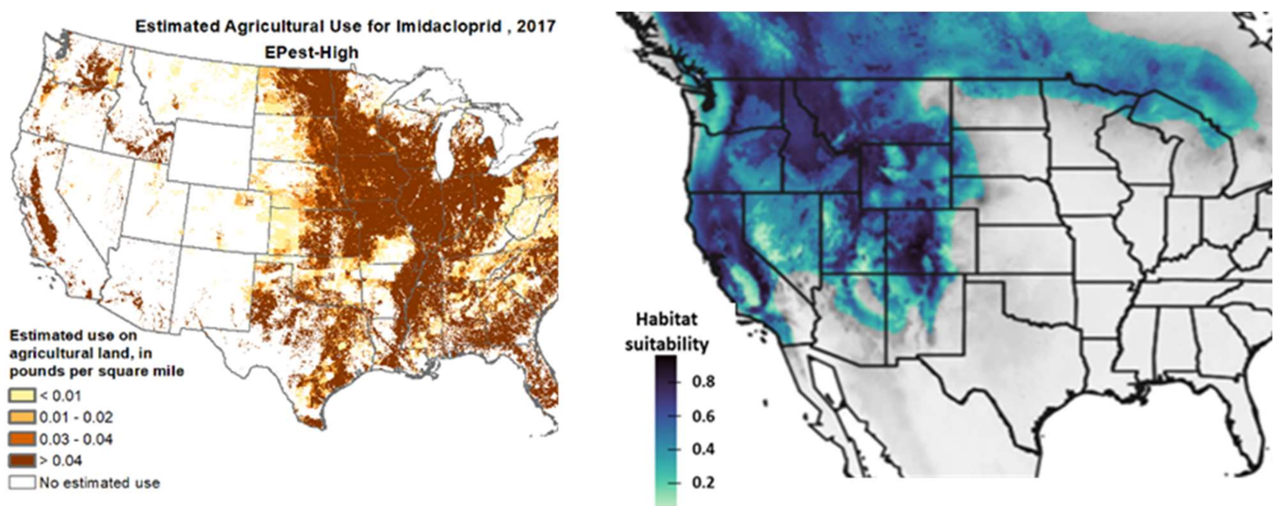

Figure 13. Imidacloprid use in the United States in 2017 (the most recent year that finalized data are available) on the left, and *E. ausonides* habitat suitability on the right. Data from USGS Pesticide National Synthesis project, available at USGS (2022).

Insect Growth Regulators

Insect Growth Regulators (IGR) are a particularly worrying class of pesticides for native lepidopteran species as these chemicals affect growth and development of larval insects, generally preventing them from reaching maturity. Insect Growth Regulators are used across metropolitan, natural, and agricultural landscapes, and are often used to manage Lepidopteran pests (Sial & Brunner 2010; El-Sheikh & Aamir 2011; Martínez et al. 2021). In one study, four IGRs, fenoxycarb, methoxyfenozide, pyriproxyfen, and tebufenozide, were tested on the moth species *Euprosterna elaeasa* and resulted in mortality between 55 and 60% (Martínez et al. 2021). The sublethal consequences of IGRs on lepidoptera are also severe: chlorfluazuron, tebofenozoid, and pyriproxyfen at all tested concentrations reduced longevity, fecundity, and fertility in the moth *Spodoptera littoralis* (Abdel-Aal 2012).

Some IGR pesticides have been demonstrated to be detrimental to Pieridae relatives of *Euchloe ausonides*. Davis et al (1991) found that second instar *Pieris brassicae* larvae on plants at various distances downwind from diflubenzuron backpack sprays saw 95% mortality up to 16 meters away at windspeeds of 5.3 m/s. Labels recommend application under less windy conditions, but even under allowable conditions, 24% of larvae died up to 24 meters away from the origin of the spray (Davis et al. 1991). This study demonstrates the risk of drift of diflubenzuron from a backpack spray, and suggests drift from airplane sprays is likely to affect nearby *E. ausonides* larvae.

Insect Growth Regulators have been found in natural environments, increasing exposure of *Euchloe ausonides* populations that are distant from the intended application. Halsch et al. (2020) found the IGR, methoxyfenozide, in 96% of the samples of milkweeds from the Central Valley of California in agriculture, wildlife refuges, metropolitan areas, and retail settings. Methoxyfenozide and tebufenozide are used as selective insecticides for Lepidopteran pests, because they bind to a receptor found in butterflies but not bees. This is a key pesticide exposure route for *E. ausonides* larvae, especially in agricultural regions in the Central Valley of California and throughout Oregon and Washington (Fig. 14) (LaLone et al. 2014).

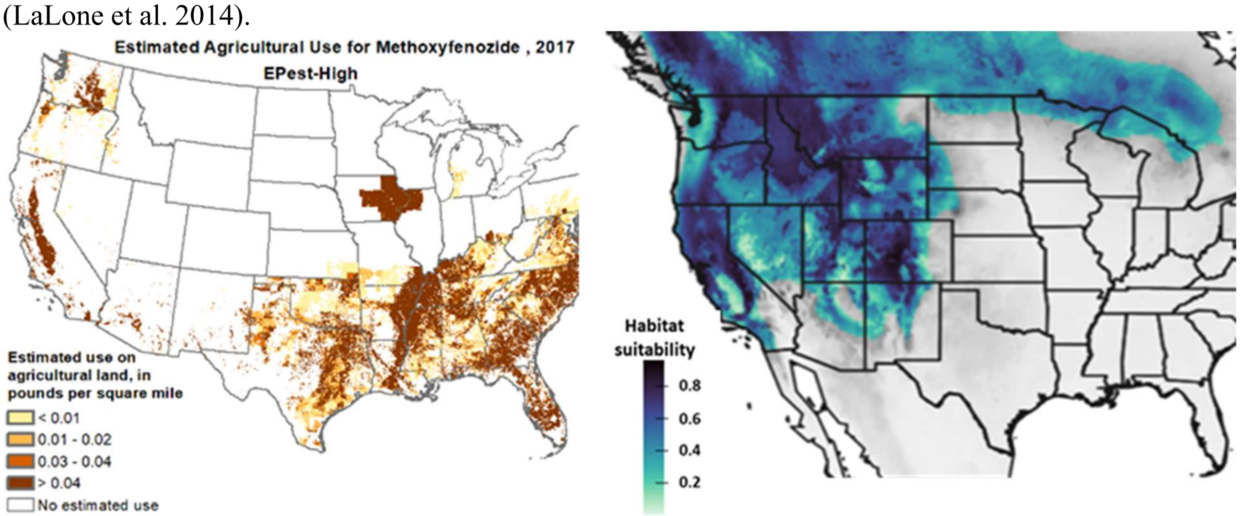

Figure 14. Methoxyfenozide use in the United States in 2017 (the most recent year that finalized data are available) on the left, and *E. ausonides* habitat quality on the right. Data from USGS Pesticide National Synthesis project, available at USGS (2022).

While IGRs are used in multiple sectors, there have been major applications of one IGR across western rangelands. Across the West, insecticides have been administered aerially by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) to prevent grasshoppers from competing with livestock for forage. On average between 2006 and 2017, APHIS administered treatments on 500,000 acres each year (Fig. 15) (Animal and Plant Health Inspection Service 2020*b*). These treatments could expose *Euchloe ausonides transmontana* and *E. a. coloradensis* to harmful levels of pesticides as with deposition of insecticide particles directly on individual larvae, or on host plants that larvae consume.

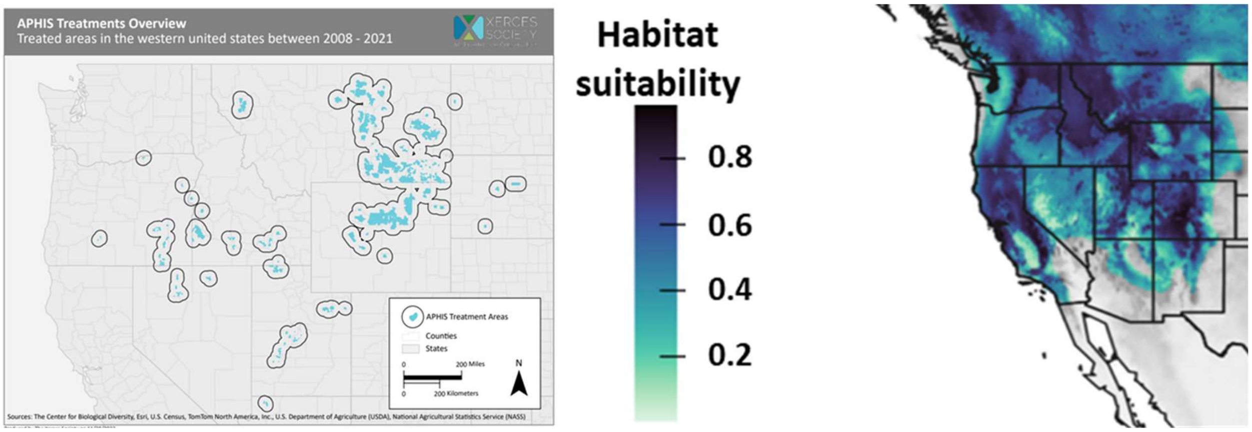

Figure 15. Aerial pesticide treatments for grasshopper control by APHIS (left), Habitat suitability (and approximate range) of *E. ausonides* (right). Applications typically use the IGR diflubenzeron and occasionally the carbamate pesticide carbaryl.

Diflubenzuron is an IGR that disrupts normal development by interfering with chitin synthesis, and was put on the market to treat larvae of the defoliator moth *Lymantria dispar* in the 1970s (Eisler & U.S. Fish and Wildlife Service 1992). Diflubenzuron is aerially applied for grasshopper suppression, with a modeled application rate of deposition on host plants between 980 and 1,760 ppb, and it can take weeks for the pesticide to degrade (Animal and Plant Health Inspection Service 2020*b*). These concentrations have been shown to be lethal to a number of lepidopteran larvae, including Pieridae relatives of *Euchloe ausonides*. In *L. dispar dispar*, all larvae died when feeding on 100 ppb diflubenzuron in diet (Eisler 1992). In *Mamestra brassicae*, 90% of larvae died when sprayed with 2,200 ppb diflubenzuron solution (Eisler 1992). In *Pieris brassicae*, a Pieridae relative of *E. ausonides*, 50% of larvae died after being sprayed with 390 ppb diflubenzuron solution (Eisler & U.S. Fish and Wildlife Service 1992). These lab studies indicate potentially high toxicity of diflubenzuron to Pierid larvae including *E. ausonides*.

Carbamates and Organophosphates

Carbamates and organophosphates are broad spectrum contact poisons that target the nervous system in insects, are used in many sectors, and can be harmful to many beneficial insects. Carbaryl is a carbamate insecticide included in 190 distinct pesticide products for use in agriculture, home gardens, on lawns, and on ornamental plants (Fig. 16) (Bond et al. 2016). Within *Euchloe ausonides ausonides'* range in California, 292 thousand pounds of carbaryl products were used in 2018 (CA DPR 2022). Carbaryl is also used in grasshopper suppression across the West (Figure 15). The carbaryl spray treatment is aerially applied by APHIS, with a modeled application rate of deposition between 37,300 and 55,000 ppb (USDA-APHIS 2019 FEIS). This chemical has been shown to have lethal and sublethal effects on lepidopteran adults; at the highest application rate under the grasshopper suppression program, more than 70% of exposed male *Cydia pomonella* (the coddling moth, which is considered an agricultural pest) and 30% of exposed females died within 24 hours (Abivardi et al. 1999). Carbaryl also exhibited high ovicidal activity in that study.

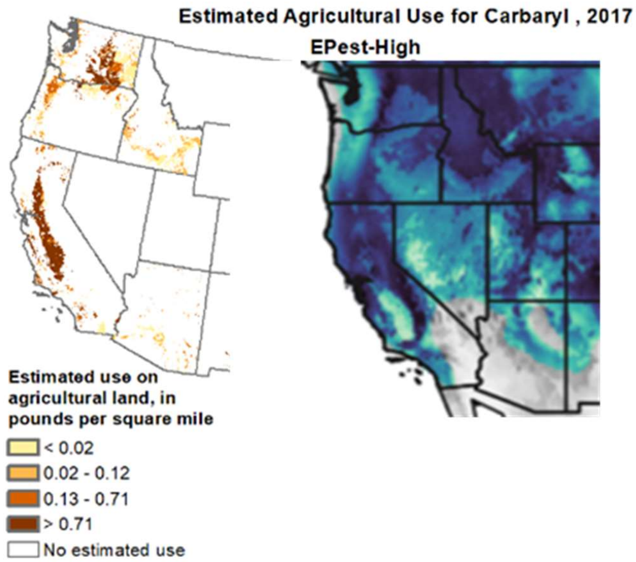

Figure 16. Carbaryl use in the United States in 2017 (most recent year that finalized data are available) on the left, and *E. ausonides transmontana* and *E. a. coloradensis* habitat quality on the right; darker colors are higher quality habitat. Data from USGS Pesticide National Synthesis project, available at USGS (2022).

Carbamates have also been shown to be very toxic to Pieridae relatives of *Euchloe ausonides*. In one study, *Pieris brassicae* host plants were sprayed with various percentages of the commercial application rate of primicarb (another carbamate) and the LD50 was identified at around 30% of the commercial application rate. This strongly suggests that users spraying at the allowable rate could have lethal effects to *E. ausonides* individuals present near pesticide applications (Braak et al. 2018 Supplementary Table 1).

While organophosphates are an older chemistry and have been phased out of many applications due to insect resistance and human health impacts, the remaining uses still can have damaging impacts on Lepidopteran populations. In addition to use on rangelands to suppress grasshoppers and Mormon crickets, other uses of organophosphates include mosquito control programs, where they are broadly applied and can cause severe impacts to non-target animals including butterflies. Insect community biodiversity losses across a wide range of orders have been reported where mosquito treatments are applied (Eliazar & Emmel 1991). Pesticide data from mosquito applications are not broadly reported, but spraying is common in many areas that have experienced substantial declines in *E. ausonides* populations, such as San Joaquin County, California and Boulder County, Colorado (Boulder County Mosquito Control District 2022; San Joaquin County Mosquito & Vector Control District 2023).

Diamides

Diamide insecticides bind to the ryanodine receptor in insects, affecting calcium regulation in muscles, and causing paralysis and death. Chlorantraniliprole is a diamide used in various sectors. Recently, it has been incorporated into seed coatings in agriculture, and it has been considered for aerial applications in grasshopper suppression efforts as well (Animal and Plant Health Inspection Service 2019*b*). Chlorantraniliprole is a concerning chemical for *Euchloe ausonides'* populations because it has long persistence time, high toxicity, and high prevalence within the butterfly's range. Chlorantraniliprole can persist in the soil for long periods, with a reported half-life up to 924 days in soils and up to 1,130 days on bare soil (Animal and Plant Health Inspection Service 2019*b*). This long persistence time increases exposure to Lepidoptera such as *E. ausonides* when it occurs on Brassicaceae plants that are larval food sources near agricultural applications, or when larvae "wander" from host plants before pupation.

Chlorantraniliprole has high toxicity in Lepidoptera: in the moth *Spodoptera cosmioides*, just 54 ppb of chlorantraniliprole killed 50% of larvae, and adults had reduced fecundity (Lutz et al. 2018). In both *Agrotis ipsilon* and *Spodoptera litura*, two other species of moths, 1,000 ppb of chlorantraniliprole killed 100% of larvae in 24 hours (Liu et al. 2017). In *Cydia pomonella*, 888 ppb chlorantraniliprole killed 90% of larvae (Bosch et al. 2018). Krishnan et al. (2020) found that the acute exposure of *Danaus plexippus* caterpillars to chlorantraniliprole was high enough to kill almost all larvae at field application rates for soybean aphids. Krishnan et al. (2021) expanded on this and found that chlorantraniliprole was between 50 and 500 times more toxic than neonicotinoids to *Danaus plexippus* larvae; based on LC_{50} rates of chlorantraniliprole to *Danaus plexippus* caterpillars, the authors' models predicted that almost all caterpillars would be killed up to 60 meters downwind from the field edge in aerial or boom applications to control soybean aphids, even after accounting for chemical degradation over time. Chlorantraniliprole was detected in 91% of samples in a recent milkweed sampling study in the Central Valley of California (Halsch et al. 2020), where *E. ausonides ausonides* occurs, and the concentrations exceeded the LD50 for monarch caterpillars in 26% of all samples, and exceeded the LD10 in 78% of all samples. Based on the chlorantraniliprole levels found in Halsch et al. (2020), Krishnan et al. (2021) estimated that the combined mean concentration across sites would cause up to 97% mortality of larvae consuming milkweed downwind of the application. The prevalence and rates of chlorantraniliprole present in the Central Valley suggest that a similarly large proportion of Brassicaceae plants used as *E. a. ausonides* larval food sources may also be contaminated to the point of lethality.

Within *Euchloe ausonides ausonides'* range in California, 364 thousand pounds of chlorantraniliprole products were used in 2018 (CA DPR 2022). Application rates are also high in northern Oregon and central Washington (Fig. 17). Toxicity studies of chlorantraniliprole on the closely related butterfly *Pieris brassicae* found high toxicity, with sprayed host plant leaves after 21 days still killing almost all larvae after five days of feeding (Su et al. 2017). We suggest that this compound would likely have a similar effect on *E. ausonides*, and as such, it poses a major threat to the continued existence of this butterfly, and especially the type subspecies *E. ausonides ausonides*. Combined with its long half-life un the soil, this pesticide's long persistence time on leaves means that it will remain toxic in the environment for long periods of time before degrading enough to decrease lethality to butterfly larvae.

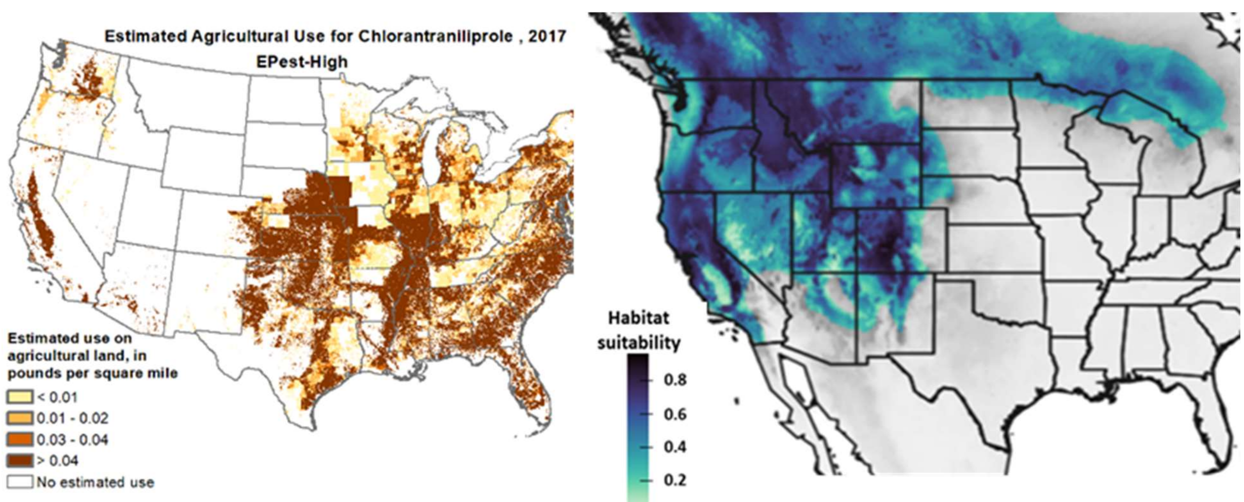

Figure 17. Chlorantraniliprole use in the United States in 2017 (the most recent year that finalized data are available) on the left, and *E. ausonides transmontana* and *E. a. coloradensis* habitat quality on the right. Data from USGS Pesticide National Synthesis project, available at USGS (2022).

Pyrethroids

Pyrethroids are more stable and persistent synthetic versions of nervous system toxins found in *Chrysanthemum* flowers, and have documented high toxicity to a broad spectrum of insects, including Pieridae relatives of *Euchloe ausonides*. Dhingra et al. (2008) evaluated the toxicity of various synthetic pyrethroids sprayed on the closely related *Pieris brassicae* larvae and ordered the chemicals' toxicity with deltamethrin as the most toxic, followed by lambda-cyhalothrin. This study also compared the LD_{50} for *P. brassicae*, *Spodoptera litura*, and *Spilarctia obliqua*, and found that *P. brassicae* was the most susceptible to these pyrethroids. Up to 75,000 pounds of lambda-cyhalothrin were applied in agricultural regions of California in 2017 alone and up to 140,000 pounds were applied in 2017 in the other western states that *E. ausonides* occupies; this chemical is used to control numerous pests and has the largest amounts applied to orchard crops (Fig. 18) (Weiben 2019; Pscheidt et al. 2020). Lambda-cyhalothrin and other pyrethroid pesticides are applied at rates that pose major risks to honey bees and other pollinators like *E. ausonides* that are exposed to pesticide drift (Riedl et al. 2006).

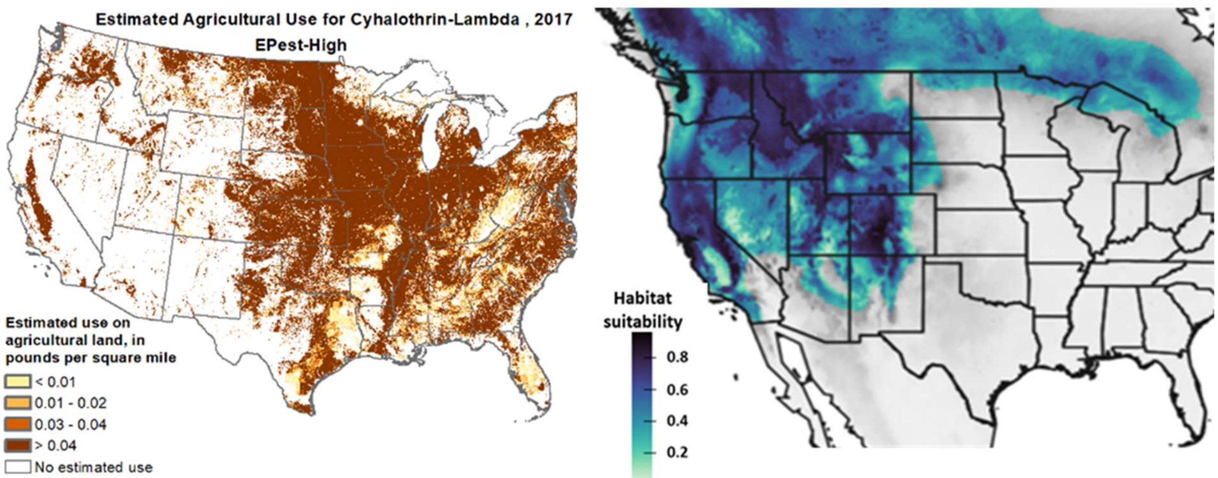

Figure 18. Agricultural application of lambda-cyhalothrin in the United States in 2017 (left, the most recent year final data is available) and *Euchloe ausonides* habitat quality (right). Data from USGS Pesticide National Synthesis project, available at USGS (2022).

Bifenthrin is a synthetic pyrethroid used in both agricultural and residential settings. One study found that bifenthrin was detected in all city creeks sampled across California (Holmes et al. 2008). This study spurred CA efforts to restrict bifenthrin and other pyrethroids out of concern for environmental contamination. However, within *Euchloe ausonides ausonides'* range in California, 911,000 pounds of bifenthrin products were still used in 2018 for both agriculture and mosquito control programs (CA DPR 2022). These chemicals similarly put *E. ausonides* at risk from drift wherever they are present near mosquito abatement treatments (Hoang & Rand 2015).

Bacillus thuringiensis var. kurstaki

Bacillus thuringiensis var. *kurstaki* (*Btk*) is a strain of bacteria with insecticidal properties, especially for Lepidoptera, and is used in agriculture, cities, and natural areas. In agriculture, *Btk* is often used to manage populations of *Lymantria dispar* and *Epiphyas postvittana*, as well as *Pieris brassicae* caterpillars, Pieridae relatives of *Euchloe ausonides*, on Brassicaceae crops (Braak et al. 2018). This could translate to high risk to *Euchloe ausonides'* subspecies, as they are related and feed on the same family of host plants. Within the range of *E. a. ausonides*, 61,000 pounds of *Btk* were used in 2018 (CA DPR 2022). Btk has also been sprayed aerially throughout the western US to protect forests from defoliators *Lymantria dispar* and *Choristoneura occidentalis* (Braak et al. 2018). Frankenhuyzen et al. (2000)

estimated a cumulative total of 8.5 million hectares (over 21 million acres) were treated in Canada and 3 million hectares (over 7.4 million acres) were treated in the US in the 1990s. *Btk* applications threaten *Euchloe ausonides* populations near forested areas within the distributions of multiple subspecies, including *E. a. transmontana* in Oregon and Washington and *E. a. mayi* populations across Canada (Boulton 2004; Hedstrom et al. 2018; Man-Son-Hing 2021; Linton & Norris 2021; Arrais 2022).

Insecticides in residential and metropolitan areas

While many pesticides are applied in agriculture, cities, and natural areas, 25% of US insecticide use is attributed to home and garden use (Atwood & Paisley-Jones 2017). Home gardens can be refuges for butterflies like *Euchloe ausonides*, but Muratet and Fontaine (2015) found that insecticides and herbicides reduced butterfly abundance in home gardens. The EPA estimates that 88 million households in the US used pesticides between 2008 and 2012 (Atwood & Paisley-Jones 2017). In many cases, application rates of pesticides like the neonicotinoid imidacloprid can be much higher in residential settings compared to agricultural rates; in some cases, these rates can result in pollen and nectar residue levels in ornamental plants that exceed the lethal concentration (LC_{50}) for honey bees (*Apis mellifera*) (Hopwood et al. 2016). These concentrations are very likely to cause sublethal or lethal effects on *E. ausonides* that feed on leaves or that consume nectar from contaminated plants. In addition, many nonnative Brassicaceae host plants are seen as weeds in residential settings and sprayed with herbicide, and *E. ausonides* larvae could feed on garden plants and be sprayed with insecticides if homeowners and gardeners mistake them for pest lepidoptera such as *Pieris rapae*.

Pesticide use data in metropolitan areas are scarce, but the USGS has collected data on pesticides in city streams across the US. Comparisons of these data from 39 states and Washington, D.C. to EPA Aquatic Life Benchmark thresholds found that pesticides in samples taken in California, Oregon, and Washington had the highest chronic risks to aquatic life (Stehle et al. 2019). These data indicate that pesticide exposure likely threatens urban populations of *Euchloe ausonides ausonides, E. a. transmontana*, and *E. a. coloradensis*.

Herbicides

Herbicides and fungicides are heavily used in agriculture (564 million pounds and 53 million pounds of active ingredient in 2012 respectively, according to Atwood and Paisley-Jones 2017). While herbicides and fungicides are not designed to target insects, they can still have harmful effects on Lepidoptera, could be contributing to the declines of *Euchloe ausonides* populations, especially near agricultural land, and pose a threat to this species.

As discussed above under Factor One in the Caterpillar Food Plant and Adult Nectar Plant Removal and Herbicide Use section, one primary threat of herbicide use to *Euchloe ausonides* populations is the removal of available caterpillar and adult food sources required by *E. ausonides* individuals. In addition, herbicidal components can also have unintended lethal or sublethal effects on Lepidoptera. Various lab studies have found lethal effects of herbicides to Lepidoptera (Kutlesa & Caveney 2001; Gupta & Bhattacharya 2008; Russell & Schultz 2010; Stark et al. 2012; Schultz et al. 2016). Herbicides can also cause sublethal effects such as changes in mass of larvae and pupae, development time, adult wing and abdomen size, fecundity, and behavior (Gupta & Bhattacharya 2008; Russell & Schultz 2010; LaBar & Schultz 2012; Stark et al. 2012; Bohnenblust et al. 2013; Schultz et al. 2016; Doll et al. 2022).

Fungicides

While there is less research on fungicide impacts on Lepidoptera, there is some evidence that fungicides can cause lethality (Siddartha & Revanna Revannavar 2014; Woods & Gent 2014; El-Kholy et al. 2018; Nicodemo et al. 2018). Fungicides can also have synergy with insecticides resulting in higher mortality in Lepidoptera (Siddartha & Revanna Revannavar 2014). In one study, virtually every Lepidopteran performance measure was affected by the fungicides azoxystrobin and tebuconazole in the lepidopteran

Spodoptera littoralis: reduced feeding, reduced larval weight, increased larval and pupal development time, increased larval mortality, reduced pupation success, reduced adult emergence, reduced fecundity and fertility, increased sterility, and reduced adult longevity (El-Kholy et al. 2018). However, effects of fungicides can be complex: *Danaus plexippus* caterpillars exposed to the fungicides azoxystrobin, pyraclostrobin, and trifloxystrobin had smaller adult sizes but lived longer than controls (Olaya-Arenas et al. 2020). Larvae of painted lady butterfly *Vanessa cardui* that were exposed to pyraclostrobin also had increased development times and smaller mass compared to controls (Peterson et al. 2019). We expect that fungicides similarly negatively affect fitness-related traits like development time, mortality, fecundity and body size in *Euchloe ausonides* and threaten populations adjacent to agricultural regions that use these chemicals.

Fungicides are commonly used in agriculture and are also found in natural landscapes some distance from their use, potentially threatening *Euchloe ausonides* populations wherever they are used. Some of the top fungicide uses in 2018 in California within the range of *E. a. ausonides* are azoxystrobin with 447 thousand pounds of product, pyraclostrobin with 302 thousand pounds of product, and tebuconazole with 193 thousand pounds of product (CA DPR 2022). In Halsch et al. (2020), azoxystrobin was found in 89% of milkweed (*Asclepias*) samples in the Central Valley of California (with 49% of the samples exceeding a sublethal level for monarch caterpillars), pyraclostrobin was found in 29% of samples, and tebuconazole was found in 26% of samples.

Climate Change

Anthropogenically driven climate changes threaten *Euchloe ausonides* populations across the species' entire range. Documented changes in climate variables across the western United States and Canada, including all of *E. ausonides'* range, include increased average surface and soil temperatures as well as reduced rainfall, reduced snowpack, increased aridity, lengthening and more frequent heatwaves, increased spread of introduced plant species, lengthened fire season, and prolonged droughts (Qian et al. 2011; Abatzoglou & Kolden 2011; Fincher 2012; Abatzoglou & Williams 2016; Vose et al. 2017; Perkins-Kirkpatrick & Lewis 2020; Pörtner et al. 2022).

Declines in moth and butterfly populations have been correlated with changes in climate, especially warming temperatures and reduced precipitation, in multiple studies across habitats and climates (Forister et al. 2010, 2021; Kucherov et al. 2021; Henry et al. 2022). Some studies have found that recent changes in climate have an influence almost equal to that of habitat loss (Forister et al. 2011; Fox et al. 2014; Halsch et al. 2021; Warren et al. 2021). Montane species have been shown to be especially vulnerable to increased temperature or prolonged drought, based on a California study during the millennial drought from 2011-2015 that included sites with *Euchloe ausonides ausonides* and *E. ausonides transmontana* (Forister et al. 2018).

Changes in climatic variables can negatively affect individual butterflies at all life stages. Recent work investigating the effects of increased heat and decreased humidity found that mortality of multiple butterfly species increased with increased heat and decreased humidity (Klockmann & Fischer 2017). Klockmann and Fisher (2017) also found that even closely related butterfly species can vary in their ability to withstand increased heat or decreased humidity. Depending on the species, different life stages may be more or less vulnerable to heat stress, and that the egg stage of three *Lycaena* species was more sensitive to these changes than early instar larvae. However, effects of heat stress on the egg stage were also shown to affect later life stages, affecting fitness traits including survival and adult mass (Klockmann et al. 2017). Other studies have found that reduced plant growth in drought years was correlated with reduced host plant occupancy by caterpillars, and that this increased the chances of metapopulation extinction in the future (Piessens et al. 2009; Johansson et al. 2020). Other plant traits, including leaf size, also affect the development and survival of lepidopteran eggs under increased temperatures (Potter et al. 2009). Negative effects of climate change to sensitive life stages, such as reduced overwintering survival, may even outweigh positive effects at other life stages like decreased larval development times (Radchuk et al. 2013).

Recently, Forister et al. (2021) found declines in abundance of most of 272 widespread western butterfly species, including *Euchloe ausonides*. Using Bayesian Poisson regression to model trends in long term butterfly monitoring data across the United States, these declines were estimated to be a continual 1.6% decline in abundance per year. These declines in abundance of butterfly communities were found to be primarily correlated with warming temperatures during the fall season; the authors hypothesize this may be due to increased physiological stress on both plants and butterflies during this time. This finding also echoes results of steep insect declines in warming parts of Europe (Outhwaite et al. 2022). More ongoing work based on the same count datasets in Forister et al. (2023) highlights the regional risk for butterfly communities in the western United States, including most of *E. ausonides'* range (Fig. 19).

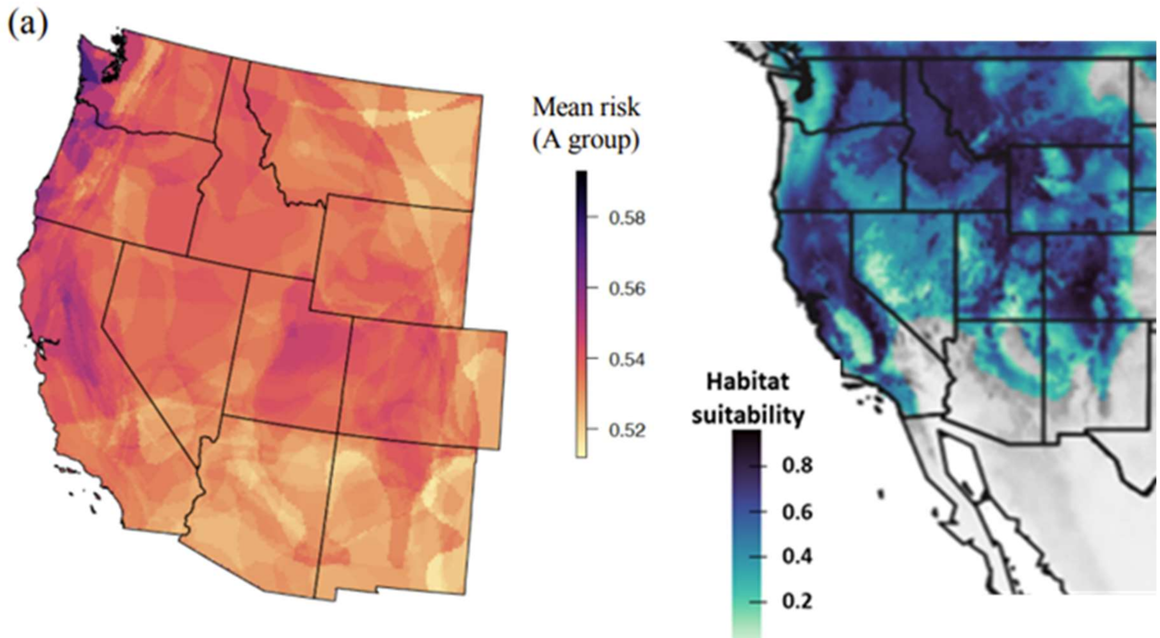

Figure 19. (Left) Index of risk for butterfly species with long term monitoring data ("A group" in this figure) across the western United States, taken from Forister et al. (2022). Each species used in risk analyses (n=184 species with monitoring data) was assigned a quantitative assessment of risk of decline in the future. Values on the map are the average risk across species found in a given area; darker values indicate higher average risk of decline for the butterfly community in that area. (Right) Habitat suitability model for *Euchloe ausonides* in the continental United States (Grames et al. in prep.)

These association of increased temperatures and decreased precipitation with long-term declines in *Euchloe ausonides*' abundance over decades constitutes an ongoing threat to the large marble butterfly across its entire range. Increases in temperature and decreases in precipitation are especially severe in the western United States, including most of *E. ausonides'* range in the United States (Cook et al. 2015; Vose et al. 2017). Reduced plant growth and vegetation die-offs are likely to include species that *E. ausonides* uses as caterpillar and adult food sources, threatening the ability of all life stages to survive. Drought has been shown to contribute to vegetation mortality in the southwestern United States, and drought levels are expected to reach levels unseen in the last 1,000 years by 2050 (Breshears et al. 2005; Park Williams et al. 2012; Munson et al. 2015; Hartmann et al. 2022). In more northern climates like those found in Alaska and the Arctic, increased air temperatures and drier soils have resulted in reduced species richness, among other vegetation changes (Hinzman et al. 2005).

Lost Genetic Diversity

With reduced abundances across its range, documented extirpations in multiple regions, increasing habitat loss and fragmentation, predation from introduced parasitoids, mortality and reduced fitness from pesticide exposure, and stress from climate changes, population sizes of *Euchloe ausonides* have been greatly reduced from historical levels. Reduced population sizes often result in reduced genetic diversity, even in widespread and mobile animals (Ripperger et al. 2013; Jangjoo et al. 2016). In fact, widespread species that have an intermediate level of specialization, such as *E. ausonides*, may actually suffer more severe losses to their population genetic diversity as fragmentation increases compared to specialists that are more accustomed to lower gene flow (Habel & Schmitt 2018). In addition, in some forested areas in the western United States fire suppression has also been shown to reduce genetic diversity in two butterfly species because of its effects on habitat fragmentation and connectivity (Gates et al. 2021); forest-associated populations of *E. ausonides* may be similarly impacted.

Conclusion

The large marble butterfly, *Euchloe ausonides*, is disappearing across its range with multiple documented extirpations, monitoring sites with reduced abundance in recent decades, and evidence of reduced abundance throughout its range based on museum and photographic records. Despite being geographically widespread, historically common, and using plants that are seemingly common on the landscape as larval food sources, the threats to larvae, adults, and their habitats jeopardize populations across the species' range in North America. Without protection, populations of *E. ausonides* will continue to disappear and abundances will continue to decline, threatening a species that ranges from Alaska to southern California with extinction. Based on the scientific evidence presented in this petition, which outlines how four of the five listing factors impact the persistence of this species, we request that the U.S. Fish and Wildlife Service act to list *Euchloe ausonides* as Threatened under the Endangered Species Act. In addition, it is clear that the type subspecies of the large marble butterfly, *E. ausonides ausonides*, needs immediate protection as an Endangered species. This subspecies has already experienced a significant reduction in range, and remaining populations are threatened by habitat destruction and modification, introduced predators, pesticide use, and effects of climate change.

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Appendix A. Sources for *Euchloe ausonides* maps and occurrence data

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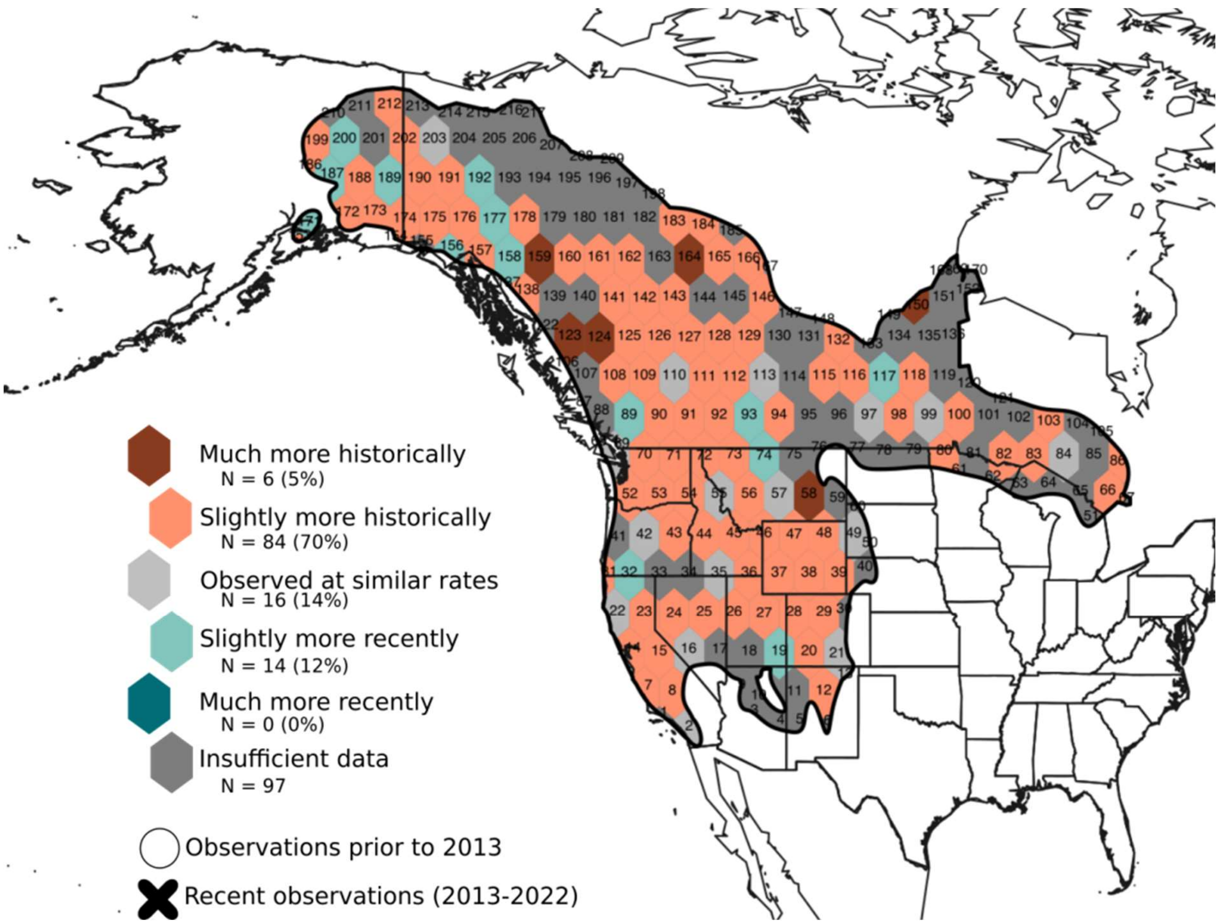
Appendix B. Full list of records used in *Euchloe ausonides* occurrence comparison.

Each numbered cell on the map corresponds to the appropriately numbered row in the table below. Columns include the total number of butterfly records in the Global Biodiversity Information Facility (GBIF) database previous to 2013 and from 2013 to present; the number of *E. ausonides* records in the GBIF database for each of those time periods; the corresponding proportion of GBIF records that *E. ausonides* make up for these time periods; and the change in relative observations between these two time periods.



Cell Number	All butterfly GBIF records <2013	All butterfly GBIF records ≥2013	*Euchloe ausonides* records <2013	*Euchloe ausonides* records ≥2013	Proportion <2013	Proportion ≥2013	Change in relative observations
1	1255	7646	0	0	0.0%	0.0%	--
2	10853	40472	4	0	0.0%	0.0%	0.0%
3	896	389	0	0	0.0%	0.0%	--
4	1474	787	0	0	0.0%	0.0%	--
5	140	17	0	0	0.0%	0.0%	--
6	577	142	0	0	0.0%	0.0%	--
7	1380	6995	4	6	0.3%	0.1%	-0.2%
8	5607	6178	32	9	0.6%	0.1%	-0.4%
9	220	234	0	0	0.0%	0.0%	--
10	2155	3084	0	0	0.0%	0.0%	--
11	2037	217	0	0	0.0%	0.0%	--
12	5317	5444	16	2	0.3%	0.0%	-0.3%
13	101	0	0	0	0.0%	--	--
14	8689	61622	141	206	1.6%	0.3%	-1.3%
15	6728	12203	19	13	0.3%	0.1%	-0.2%
16	1028	739	2	1	0.2%	0.1%	-0.1%
17	771	266	0	0	0.0%	0.0%	--
18	1663	921	0	1	0.0%	0.1%	--
19	1036	2068	5	15	0.5%	0.7%	0.2%
20	4720	2077	63	12	1.3%	0.6%	-0.8%
21	3184	2869	15	11	0.5%	0.4%	-0.1%
22	1305	3355	1	1	0.1%	0.0%	0.0%
23	4784	10854	24	9	0.5%	0.1%	-0.4%
24	426	175	4	1	0.9%	0.6%	-0.4%
25	687	414	2	0	0.3%	0.0%	-0.3%
26	1438	414	14	1	1.0%	0.2%	-0.7%
27	6632	3071	80	14	1.2%	0.5%	-0.8%
28	1497	823	16	5	1.1%	0.6%	-0.5%
29	15831	17350	212	125	1.3%	0.7%	-0.6%
30	227	203	0	0	0.0%	0.0%	--
31	334	856	2	2	0.6%	0.2%	-0.4%
32	1915	4139	7	27	0.4%	0.7%	0.3%
33	885	1662	0	9	0.0%	0.5%	--
34	312	108	0	0	0.0%	0.0%	--
35	1091	407	1	0	0.1%	0.0%	-0.1%
36	3706	2753	39	14	1.1%	0.5%	-0.5%
37	1061	399	10	0	0.9%	0.0%	-0.9%
38	1248	243	10	0	0.8%	0.0%	-0.8%

Cell Number	All butterfly GBIF records <2013	All butterfly GBIF records ≥2013	*Euchloe ausonides* records <2013	*Euchloe ausonides* records ≥2013	Proportion <2013	Proportion ≥2013	Change in relative observations
39	1763	1310	8	2	0.5%	0.2%	-0.3%
40	192	478	0	0	0.0%	0.0%	--
41	1745	3319	0	0	0.0%	0.0%	--
42	1602	1979	1	1	0.1%	0.1%	0.0%
43	749	581	7	0	0.9%	0.0%	-0.9%
44	3394	1503	39	1	1.1%	0.1%	-1.1%
45	2179	3034	23	3	1.1%	0.1%	-1.0%
46	3839	2189	46	17	1.2%	0.8%	-0.4%
47	2041	442	16	3	0.8%	0.7%	-0.1%
48	942	267	6	0	0.6%	0.0%	-0.6%
49	3363	549	10	2	0.3%	0.4%	0.1%
50	9	7	0	0	0.0%	0.0%	--
51	123	117	0	0	0.0%	0.0%	--
52	1222	5328	5	0	0.4%	0.0%	-0.4%
53	1258	1153	38	5	3.0%	0.4%	-2.6%
54	7393	1941	155	4	2.1%	0.2%	-1.9%
55	1795	1733	5	4	0.3%	0.2%	0.0%
56	892	451	26	3	2.9%	0.7%	-2.2%
57	2522	393	28	4	1.1%	1.0%	-0.1%
58	130	10	8	0	6.2%	0.0%	-6.2%
59	267	81	0	1	0.0%	1.2%	--
60	18	0	0	0	0.0%	--	--
61	11	6	0	0	0.0%	0.0%	--
62	150	340	0	0	0.0%	0.0%	--
63	512	226	0	0	0.0%	0.0%	--
64	596	496	0	0	0.0%	0.0%	--
65	3395	7830	0	0	0.0%	0.0%	--
66	911	4806	4	0	0.4%	0.0%	-0.4%
67	64	358	0	0	0.0%	0.0%	--
68	1	13	0	0	0.0%	0.0%	--
69	6010	10689	6	1	0.1%	0.0%	-0.1%
70	10664	7437	88	13	0.8%	0.2%	-0.7%
71	7900	8472	129	29	1.6%	0.3%	-1.3%
72	3095	1468	30	4	1.0%	0.3%	-0.7%
73	4797	2028	46	7	1.0%	0.3%	-0.6%
74	1528	380	7	5	0.5%	1.3%	0.9%
75	722	259	0	0	0.0%	0.0%	--
76	271	232	0	0	0.0%	0.0%	--

Cell Number	All butterfly GBIF records <2013	All butterfly GBIF records ≥2013	*Euchloe ausonides* records <2013	*Euchloe ausonides* records ≥2013	Proportion <2013	Proportion ≥2013	Change in relative observations
77	178	41	0	0	0.0%	0.0%	--
78	955	282	0	0	0.0%	0.0%	--
79	1822	125	0	0	0.0%	0.0%	--
80	4802	1651	123	11	2.6%	0.7%	-1.9%
81	653	2330	0	2	0.0%	0.1%	--
82	1265	1881	21	7	1.7%	0.4%	-1.3%
83	3075	2997	40	16	1.3%	0.5%	-0.8%
84	378	579	1	1	0.3%	0.2%	-0.1%
85	293	340	0	1	0.0%	0.3%	--
86	108	565	1	0	0.9%	0.0%	-0.9%
87	0	0	0	0	--	--	--
88	352	290	0	0	0.0%	0.0%	--
89	6619	1467	35	12	0.5%	0.8%	0.3%
90	8235	4764	143	22	1.7%	0.5%	-1.3%
91	3126	3751	8	3	0.3%	0.1%	-0.2%
92	10898	6431	73	19	0.7%	0.3%	-0.4%
93	3012	1439	1	3	0.0%	0.2%	0.2%
94	1737	1106	16	0	0.9%	0.0%	-0.9%
95	365	172	0	0	0.0%	0.0%	--
96	576	704	0	0	0.0%	0.0%	--
97	3095	129	1	0	0.0%	0.0%	0.0%
98	7187	760	67	3	0.9%	0.4%	-0.5%
99	9666	5093	18	8	0.2%	0.2%	0.0%
100	1961	1354	23	2	1.2%	0.1%	-1.0%
101	267	162	0	0	0.0%	0.0%	--
102	102	37	0	0	0.0%	0.0%	--
103	1283	107	27	0	2.1%	0.0%	-2.1%
104	155	44	0	0	0.0%	0.0%	--
105	49	24	0	0	0.0%	0.0%	--
106	0	0	0	0	--	--	--
107	53	53	0	0	0.0%	0.0%	--
108	1155	416	24	0	2.1%	0.0%	-2.1%
109	527	209	1	0	0.2%	0.0%	-0.2%
110	2571	887	10	3	0.4%	0.3%	-0.1%
111	2994	642	38	0	1.3%	0.0%	-1.3%
112	4805	6899	31	8	0.6%	0.1%	-0.5%
113	1388	374	7	2	0.5%	0.5%	0.0%
114	250	75	0	0	0.0%	0.0%	--

Cell Number	All butterfly GBIF records <2013	All butterfly GBIF records ≥2013	*Euchloe ausonides* records <2013	*Euchloe ausonides* records ≥2013	Proportion <2013	Proportion ≥2013	Change in relative observations
115	1563	3428	3	2	0.2%	0.1%	-0.1%
116	222	93	3	0	1.4%	0.0%	-1.4%
117	2170	141	33	3	1.5%	2.1%	0.6%
118	475	86	2	0	0.4%	0.0%	-0.4%
119	142	30	0	0	0.0%	0.0%	--
120	94	1	0	0	0.0%	0.0%	--
121	0	0	0	0	--	--	--
122	40	9	0	0	0.0%	0.0%	--
123	136	930	10	0	7.4%	0.0%	-7.4%
124	58	198	6	0	10.3%	0.0%	-10.3%
125	1077	328	5	0	0.5%	0.0%	-0.5%
126	920	186	4	0	0.4%	0.0%	-0.4%
127	430	114	1	0	0.2%	0.0%	-0.2%
128	520	303	2	0	0.4%	0.0%	-0.4%
129	445	245	3	0	0.7%	0.0%	-0.7%
130	40	98	0	1	0.0%	1.0%	--
131	70	31	0	0	0.0%	0.0%	--
132	199	48	2	0	1.0%	0.0%	-1.0%
133	391	37	0	0	0.0%	0.0%	--
134	539	57	0	0	0.0%	0.0%	--
135	391	138	0	0	0.0%	0.0%	--
136	709	0	17	0	2.4%	--	--
137	0	0	0	0	--	--	--
138	140	13	1	0	0.7%	0.0%	-0.7%
139	26	19	0	0	0.0%	0.0%	--
140	2	0	0	0	0.0%	--	--
141	822	47	7	0	0.9%	0.0%	-0.9%
142	382	77	16	0	4.2%	0.0%	-4.2%
143	575	26	5	0	0.9%	0.0%	-0.9%
144	91	33	0	0	0.0%	0.0%	--
145	141	3	0	0	0.0%	0.0%	--
146	207	262	3	0	1.4%	0.0%	-1.4%
147	2	0	0	0	0.0%	--	--
148	0	0	0	0	--	--	--
149	77	18	0	0	0.0%	0.0%	--
150	5	3	1	0	20.0%	0.0%	-20.0%
151	36	2	0	0	0.0%	0.0%	--
152	42	10	0	0	0.0%	0.0%	--

Cell Number	All butterfly GBIF records <2013	All butterfly GBIF records ≥2013	*Euchloe ausonides* records <2013	*Euchloe ausonides* records ≥2013	Proportion <2013	Proportion ≥2013	Change in relative observations
153	110	24	1	0	0.9%	0.0%	-0.9%
154	0	0	0	0	--	--	--
155	9	0	0	0	0.0%	--	--
156	1093	57	13	1	1.2%	1.8%	0.6%
157	3268	456	117	11	3.6%	2.4%	-1.2%
158	226	43	5	1	2.2%	2.3%	0.1%
159	455	189	34	3	7.5%	1.6%	-5.9%
160	105	51	1	0	1.0%	0.0%	-1.0%
161	803	129	2	0	0.2%	0.0%	-0.2%
162	179	23	2	0	1.1%	0.0%	-1.1%
163	92	1	0	0	0.0%	0.0%	--
164	69	12	4	0	5.8%	0.0%	-5.8%
165	109	8	1	0	0.9%	0.0%	-0.9%
166	1456	45	19	0	1.3%	0.0%	-1.3%
167	1	0	0	0	0.0%	--	--
168	0	0	0	0	--	--	--
169	2071	17	0	0	0.0%	0.0%	--
170	4	0	0	0	0.0%	--	--
171	938	568	5	10	0.5%	1.8%	1.2%
172	861	16	36	0	4.2%	0.0%	-4.2%
173	869	13	10	0	1.2%	0.0%	-1.2%
174	629	137	1	0	0.2%	0.0%	-0.2%
175	3691	416	38	3	1.0%	0.7%	-0.3%
176	2506	701	66	6	2.6%	0.9%	-1.8%
177	262	29	14	2	5.3%	6.9%	1.6%
178	83	22	3	0	3.6%	0.0%	-3.6%
179	59	52	0	1	0.0%	1.9%	--
180	110	124	0	0	0.0%	0.0%	--
181	125	4	0	0	0.0%	0.0%	--
182	217	31	0	0	0.0%	0.0%	--
183	99	5	1	0	1.0%	0.0%	-1.0%
184	390	26	7	0	1.8%	0.0%	-1.8%
185	40	5	0	0	0.0%	0.0%	--
186	59	5	2	0	3.4%	0.0%	-3.4%
187	1994	1056	118	67	5.9%	6.3%	0.4%
188	2532	87	32	1	1.3%	1.1%	-0.1%
189	2333	60	25	1	1.1%	1.7%	0.6%
190	1196	150	44	1	3.7%	0.7%	-3.0%

Cell Number	All butterfly GBIF records <2013	All butterfly GBIF records ≥2013	*Euchloe ausonides* records <2013	*Euchloe ausonides* records ≥2013	Proportion <2013	Proportion ≥2013	Change in relative observations
191	1343	131	28	1	2.1%	0.8%	-1.3%
192	645	71	12	2	1.9%	2.8%	1.0%
193	170	2	0	0	0.0%	0.0%	--
194	83	2	0	0	0.0%	0.0%	--
195	38	0	0	0	0.0%	--	--
196	136	0	0	0	0.0%	--	--
197	0	0	0	0	--	--	--
198	0	0	0	0	--	--	--
199	16056	694	336	6	2.1%	0.9%	-1.2%
200	4141	139	8	1	0.2%	0.7%	0.5%
201	283	6	0	0	0.0%	0.0%	--
202	496	10	4	0	0.8%	0.0%	-0.8%
203	6761	141	5	0	0.1%	0.0%	-0.1%
204	3114	41	0	0	0.0%	0.0%	--
205	2	3	0	0	0.0%	0.0%	--
206	1	0	0	0	0.0%	--	--
207	256	0	0	0	0.0%	--	--
208	83	0	0	0	0.0%	--	--
209	0	0	0	0	--	--	--
210	0	0	0	0	--	--	--
211	583	0	1	0	0.2%	--	--
212	634	2	3	0	0.5%	0.0%	-0.5%
213	274	8	0	0	0.0%	0.0%	--
214	144	32	0	0	0.0%	0.0%	--
215	165	11	0	0	0.0%	0.0%	--
216	12	0	0	0	0.0%	--	--
217	0	0	0	0	--	--	--